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CONTENTS OF VOLUME 34

JULY 1942. NUMBER 1

OPIE NUMBER	PAGE
An Inquiry into Certain Aspects of Eugene L. Opie. Peyton Rous, M.D., New York.....	1
Publications of Eugene L. Opie from 1898 to 1941.....	7
Suprasellar Tumors Related to the Pars Intermedia of the Hypophysis. W. G. MacCallum, M.D., Baltimore.....	13
Influence of Various Types of Immunization on the Genesis of Experimental Hemolytic Streptococcus Arthritis. D. Murray Angevine, M.D., Wilmington, Del., and Russell L. Cecil, M.D., and Sidney Rothbard, M.D., New York.....	18
Relationship of Coccidioidomycosis to Calcified Pulmonary Nodules. Joseph D. Aronson, M.D., Philadelphia, and Robert M. Saylor, M.D., and Erma I. Parr, R.N., Washington, D. C.....	31
Effects of Progesterone on the Sex Organs and on the Production of Placenta in the Female Guinea Pig. H. T. Blumenthal, Ph.D., and Leo Loeb, M.D., St. Louis.....	49
The Pulmonary Apical Scar—An Inquiry. C. H. Bunting, M.D., Madison, Wis.	67
Standardization of Tuberculin with the Aid of Guinea Pigs Sensitized by Killed Tubercle Bacilli in Liquid Petrolatum. Jules Freund, M.D., Otisville, N. Y., and Russell Y. Gottschall, Ph.D., Lansing, Mich.....	73
The Teaching of Experimental Pathology. Jacob Furth, M.D., New York..	75
Studies of Amyloid: II. The Isolation of a Polysaccharide from Amyloid-Bearing Tissues. George Hass, M.D., New York.....	92
Use of the Supravital Staining Technic in the Study of Tumors of the Lymphosarcoma Group. C. H. Hu, M.D., and H. C. Pai, M.B., Ch.B., Peiping, China.....	106
The Spleen in the Leukemias. E. B. Krumbhaar, M.D., and Alfred Stengel, M.D., Philadelphia.....	117
Tumors of the Nerve Sheaths in Fish of the Snapper Family (Lutianidae). Balduin Lucké, M.D., Philadelphia.....	133
Effect of Chorionic Gonadotropin on the Spread of Particulate Substances in the Skin of Rabbits. Max B. Lurie, M.D., and Peter Zappasodi, B.S., Philadelphia	151
Chemotaxis. Morton McCutcheon, M.D., Philadelphia.....	167
Biochemical Factors in Inflammation and Diabetes Mellitus. Valy Menkin, M.D., Boston.....	182
Effect of Parabiosis on the Hepatic Changes Following Obstruction of the Common Bile Duct in Rats. Robert A. Moore, M.D., St. Louis; Louis M. Hellman, M.D., Baltimore, and Hermann Jacobius, M.D., New York.	196
Bacterial Morphology as Shown by the Electron Microscope: IV. Structural Differentiation Within the Bacterial Protoplasm. Stuart Mudd, M.D.; Katherine Polevitzky, M.D., and Thomas F. Anderson, Ph.D., Philadelphia.	199
Regeneration of Rat Liver at Different Ages: Metabolism of Embryonic, Neonatal and Regenerating Rat Liver. J. L. Norris, B.S.; J. Blanchard, B.A., and C. Povolny, B.A., New York.....	208
Studies of Spontaneous Tumors in Guinea Pigs: II. Tumors of the Stomach and Intestine. George N. Papanicolaou, M.D., and Charles T. Olcott, M.D., New York.....	218
Experimental Histoplasmosis in Mice. Robert J. Parsons, M.D., Ann Arbor, Mich.	229
Fibrosarcoma of Arachnoidal Origin with Metastases: Report of Four Cases with Necropsy. William O. Russell, M.D., and Ernest Sachs, M.D., St. Louis.....	240
Elastic Properties of the Rabbit Aorta in Relation to Age. John A. Saxton Jr., M.D., New York.....	262

JULY—Continued

	PAGE
Nucleoprotein Antigen of Vaccine Virus: I. A New Antigen from Elementary Bodies of Vaccinia. Joseph E. Smadel, M.D.; Thomas M. Rivers, M.D., and Charles L. Hoagland, M.D., New York.....	275
Encephalomalacia with Cavity Formation in Infants. Lewis D. Stevenson, M.D., and Lillian E. McGowan, M.D., New York.....	286
Developmental Abnormalities of the Lung and Bronchiogenic Carcinoma. Nathan A. Womack, M.D., and Evarts A. Graham, M.D., St. Louis.....	301

AUGUST 1942. NUMBER 2

Susceptibility of the Heart of the Rabbit to Specific Infection in Viral Diseases. John Musser Pearce, M.D., Brooklyn.....	319
Effect of Thyroxin and the Anterior Pituitary Growth Hormone on Endochondral Ossification. Species Used: The Rat. Hermann Becks, M.D., San Francisco; Robert D. Ray, M.A., Boston, and Miriam E. Simpson, M.D., and Herbert M. Evans, M.D., Berkeley, Calif.....	334
Secondary Tumors of the Heart. Peter A. Herbut, M.D., and Albert L. Maisel, M.D., Philadelphia.....	358
Fibrinoid Necrosis in Arteriosclerosis. Nathaniel Charles Schlossmann, M.D., New York.....	365
Skeletal Abnormalities Induced in Rats by Maternal Nutritional Deficiency: Histologic Studies. Josef Warkany, M.D., and Rose C. Nelson, Ph.D., Cincinnati	375
Clinical Significance of the Pathologic Changes in Giant Follicular Lymphadenopathy. Douglas Symmers, M.D., New York.....	385
Kaposi's Sarcoma: A Critical Survey. Ernest E. Aegerter, M.D., and Augustin R. Peale, M.D., Philadelphia.....	413
Case Reports:	
Congestive Splenomegaly (Banti's Disease) Due to Portal Stenosis Without Hepatic Cirrhosis; Aneurysms of the Splenic Artery: Report of a Case with Necropsy. W. K. Trimble, M.D., and J. H. Hill, M.D., Kansas City, Mo.....	423
Traumatic Hemorrhage into the Pituitary Gland. R. A. Munslow, M.D., Abilene, Texas; J. L. Haymond, M.D., Indianapolis, and A. S. Crawford, M.D., Detroit.....	431
Metastatic Calcification in Osteitis Deformans (Paget's Disease of Bone). H. Gideon Wells, M.D., and Sion W. Holley, M.D., Chicago.....	435
General Reviews:	
Effects of Radiation on Normal Tissues (To Be Continued). Shields Warren, M.D., Boston:	
I. Introduction	443
II. The Effects of Radiation on the Cell.....	446
Pathologic Effects Produced by Deficiency of Single Metallic and Non-metallic Elements. Richard H. Follis Jr., M.D., Baltimore.....	451
Notes and News.....	469
Book Reviews.....	470
Books Received.....	472

SEPTEMBER 1942. NUMBER 3

Carcinoma of the Prostate: Correlation Between the Histologic Observations and the Clinical Course. Newton Evans, M.D.; Roger W. Barnes, M.D., and Albert F. Brown, M.D., Los Angeles.....	473
Coccidioidomycosis in States Other Than California, with Report of a Case in Louisiana. John R. Schenken, M.D., and Emil E. Palik, M.D., New Orleans	484
Metastatic Tumors of the Nervous System. A. B. Baker, M.D., Ph.D., Minneapolis	495
Cultivation of Human Leukemic Leukocytes on the Chorioallantoic Membrane of the Chicken Egg. Mila Pierce, M.D., Chicago.....	538
Case Reports:	
Multiple Myeloma with Unusual Visceral Involvement. Jacob Churg, M.D., and Alvin J. Gordon, M.D., New York.....	546

CONTENTS OF VOLUME 34

v

SEPTEMBER—Continued

PAGE

Myocardial Lesions in Myasthenia Gravis: Review and Report of a Case. Antonio Rottino, M.D., and Robert Poppiti, M.D., New York, and John Rao, M.D., Detroit.....	557
General Reviews:	
Effects of Radiation on Normal Tissues (To Be Continued). Shields Warren, M.D., Boston:	
III. Effects of Radiation on the Blood and the Hemopoietic Tissues, Including the Spleen, the Thymus and the Lymph Nodes. Charles E. Dunlap, M.D., Boston.....	562
Notes and News.....	609
Book Reviews.....	610
Books Received.....	612

OCTOBER 1942. NUMBER 4

Comparative Pathology of Induced Tumors of the Salivary Glands. Paul E. Steiner, M.D., Ph.D., Chicago.....	613
Mechanism of Parathyroid Hormone Action. Hans Selye, M.D., Ph.D., D.Sc., F.R.S.C., Montreal, Canada.....	625
Renal Changes in the Albino Rat on Low Choline and Choline-Deficient Diets. Kermit Christensen, Ph.D., St. Louis.....	633
Variation of Vitamin A Fluorescence in the Cyclic Changes of the Ovary. Alex B. Ragins, M.D., and Hans Popper, M.D., Chicago.....	647
Comparison of Pathologic Observations in Weil's Disease and in Yellow Fever. William H. Harris Jr., M.D., New Orleans.....	663
Tumors of the Striatohalamic and Related Regions: Their Probable Source of Origin and More Common Forms. Joseph H. Globus, M.D., New York, and Hartwig Kuhlbeck, M.D., Philadelphia.....	674
Anatomic Changes in the Prostate of Patients with Cirrhosis of the Liver. S. D. Wu, M.D., St. Louis.....	735
Case Reports:	
Oxyuris Vermicularis Infection of the Wall of a Fallopian Tube. B. Chomet, M.D., Elyria, Ohio.....	742
Syphilis of the Aorta and Coronary Arteries. George Strassmann, M.D., and Philip Goldstein, M.D., New York.....	745
General Reviews:	
Effects of Radiation on Normal Tissues (To Be Continued). Shields Warren, M.D., Boston:	
IV. Effects of Radiation on the Gastrointestinal Tract, Including the Salivary Glands, the Liver and the Pancreas. Nathan B. Friedman, M.D., San Francisco.....	749
Notes and News.....	788
Book Reviews.....	789
Books Received.....	790

NOVEMBER 1942. NUMBER 5

Spontaneous Coccidioidal Granuloma in the Lungs of Wild Rodents. L. L. Ashburn, M.D., and C. W. Emmons, Ph.D., Bethesda, Md.....	791
Reduction of Pulmonary Resistance to Infection by Circulating Toxins. Douglas H. Sprunt, M.D., and Willard Camalier Jr., M.D., Durham, N. C.....	801
Elastic Tissue: I. Description of a Method for the Isolation of Elastic Tissue. George M. Hass, M.D., New York.....	807
Heart Weight: I. The Weight of the Normal Human Heart. Pearl M. Zeek, M.D., Cincinnati.....	820
Papillary Cystadenoma Lymphomatosum. Max Lederer, M.D., and David M. Grayzel, M.D., Ph.D., Brooklyn.....	833
Pancreodochocystostomy and Experimental Production of Gallstones. Hans G. Aronson, M.D., Chicago.....	843
Sudden Death Following Injection of Foreign Protein. B. M. Vance, M.D., and George Strassmann, M.D., New York.....	849

NOVEMBER—Continued

	PAGE
Bilateral Cortical Necrosis of the Kidney: A Report of Two Cases. Walter H. Sheldon, M.D., and Arthur T. Hertig, M.D., Boston.....	866
Relationship of Heart Size to Cholesterol Content in Experimental Atheromatosis of the Rabbit. M. Hurwitz, M.D., and L. Friedberg, B.S., Chicago.....	875
Experimental Studies in Cardiovascular Pathology: VI. Pectin Atheromatosis and Thesauriosis in Rabbits and in Dogs. W. C. Hueper, M.D., New York.....	883
Case Reports:	
Recklinghausen's Disease with Unusual Symptoms from Intestinal Neurofibroma. John Grill, M.D., and Joseph F. Kuzma, M.D., Milwaukee.....	902
Papilloma of the Choroid Plexus: Report of a Case and Summary of Recorded Cases. Louis C. Posey, M.D., Birmingham, Ala.....	911
General Reviews:	
Effects of Radiation on Normal Tissues (To Be Continued). Shields Warren, M.D., Boston:	
V. Effects on the Respiratory System.....	917
Forensic Medicine:	
Notes on the Identification of Seminal Stains by Means of the Florence Reaction. Joseph Beeman, M.D., Portland, Ore.....	932
Notes and News.....	934
Book Reviews.....	935
Books Received.....	936

DECEMBER 1942. NUMBER 6

Experimental Cholesterol Atheromatosis in an Omnivorous Animal, the Chick. D. V. Dauber, M.D., and L. N. Katz, M.D., Chicago.....	937
Localized Pleural Mesothelioma: Investigation of Its Characteristics and Histogenesis by the Method of Tissue Culture. Arthur Purdy Stout, M.D., and Margaret R. Murray, Ph.D., New York.....	951
A Heretofore Unrecognized Mechanical Principle Effective in Aortic Sclerosis. Joseph Krafka Jr., M.D., Augusta, Ga.....	965
Elastic Tissue: II. A Study of the Elasticity and Tensile Strength of Elastic Tissue Isolated from the Human Aorta. George M. Hass, M.D., New York.....	971
Atrophy, Degeneration and Metaplasia in Denervated Skeletal Muscle. Rudolf Altschul, M.U.Dr., Saskatoon, Sask., Canada.....	982
Arteriosclerosis Obliterans: A Study of the Lesions in Occluding Peripheral Sclerosis, with a Note on Mönckeberg's Sclerosis. S. W. Sappington, M.D., and H. Russell Fisher, M.D., Philadelphia.....	989
Heterotopic Brain Tissue in the Lungs of Two Anencephalic Monsters. Edith L. Potter, M.D., and Raymond L. Young, M.D., Chicago.....	1009
Transposition of the Aorta and the Pulmonary Artery: An Embryologic Study of Its Cause. John L. Bremer, M.D., Boston.....	1016
The Adrenal Cortex in Essential Hypertension. William S. Dempsey, M.D., Montreal, Canada.....	1031
Production of Cirrhosis of the Liver in Rats by Feeding Low Protein, High Fat Diets. Harold Blumberg, Sc.D., Baltimore, and Hugh G. Grady, M.D., Darby, Pa.....	1035
The Parathyroid Gland in Infancy. Eugene Kaplan, M.D., Boston.....	1042
Equine Encephalomyelitis (Western) in Man—A Histologic and Anatomic Study. James H. Peers, M.D., Bethesda, Md.....	1050
Case Reports:	
Acute Pancreatitis Following Blood Transfusion. Lauren V. Ackerman, M.D., Columbia, Mo.....	1065
General Reviews:	
Effects of Radiation on Normal Tissues (To Be Continued). Shields Warren, M.D., Boston:	
VI. Effects of Radiation on the Cardiovascular System.....	1070
VII. Effects of Radiation on the Urinary System.....	1079
Notes and News.....	1085
Book Reviews.....	1086
Books Received.....	1088
General Index.....	1089

SPECIAL NUMBER

DEDICATED TO

DR. EUGENE L. OPIE

**Emeritus Professor of Pathology, Cornell University
Medical College**

BY

**HIS FORMER ASSOCIATES ON THE OCCASION
OF HIS RETIREMENT**



Eugene L. Opie

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AN INQUIRY INTO CERTAIN ASPECTS OF EUGENE L. OPIE

In the long run the experimental pathologist expresses himself as surely as the artist. He does it despite the cryptic, the surprising and the negative results of his labors, the demands of his fellows that he stick to the truth when telling of these, and the ritualistic character of modern scientific reporting. A single early paper may tell little of a man, being so often the product of chance and the prepared mind of some older associate, but as years pass his researches delineate him. This is the reason why the contributors to the present number of the ARCHIVES OF PATHOLOGY honor Eugene Opie over and above the discoveries he has made. This is why they offer in praise of him their own scientific papers. And these are personal signatures in attestation of regard.

It is recorded of Dr. Opie's father that on his way south to fight for the Confederacy he stopped over in Washington to attend Lincoln's inaugural ball. One can see in this action the same intellectual curiosity tempered with humor which has proved so effective in his son. Later Thomas Opie became one of the founders of the College of Physicians and Surgeons in Baltimore, serving as dean and professor of obstetrics and gynecology throughout a long era turbulent with medical factions. Eugene Opie duly entered this college, on deciding to become a doctor, but after a year sought admission to the Johns Hopkins Medical School. He was told that the faculty had decided not to accord advanced standing to entrants. A little later though, Dr. William Henry Welch, who was then dean at the Hopkins, summoned him to say that a young Canadian had been accepted for the second year and hence in fairness he would be allowed to enter too. Thus it came about that Eugene Opie and William MacCallum were in due course among the earliest to graduate from the Johns Hopkins Medical School. While it may be true that good men cannot be kept down, certainly chance helps raise them up. Through accidents of time and circumstance Opie straightway gained teachers able to teach the art of discovery, and so swiftly did he learn his lesson that he uncovered facts of large medical import even before he graduated. Within a few months of entering the Johns Hopkins Medical School he happened on some strange looking cells in a section of pancreas and asked Dr. Welch what they were. He was told that they had been described years previously by a youngster of that day, named Langerhans, that nobody knew their significance and that it would be good to find out. From this

incident was to come our present realization of a basic relationship of the islands of Langerhans to sugar metabolism. But this was not all. W. S. Thayer, whose medical passion then was the study of malaria, suggested to both Opie and MacCallum that they look into the disease as affecting the birds about Baltimore. They found it to be prevalent in sparrows and red wing blackbirds, and scrutinizing this material, came on many new facts, which were reported in conjoined papers the year after they graduated.

There is no better way to learn how to behave as scientist than to study the moves of a man who has done well. However much one may think one knows, or feel that one has accomplished, however far learning may be separated from doing, the task remains inspiring, since through it one can perceive a larger unity than that of observation with experiment: One can see the thinker using his thought aright. With this in mind, let us now examine into the recorded scientific works of Eugene Opie.¹

It is plain from Opie's early papers that he started to discover by asking himself urgently what each and every thing meant that came under his eye. When examining postmortem material he was no recorder of final states; the changes met in the dead had for him the liveliest of implications. He was an inquirer into riddles, who sought after the meaning of diseased organs much as one might seek for the answers to charades. It was with this approach to the unknown that he observed in postmortem sections from the pancreas of a diabetic person the nearly complete destruction by hyaline change of the islands of Langerhans. The hint was enough. He had proved to himself while yet a medical student that the islands were not modified or undeveloped acinous cells, as was currently thought, and had discriminated the interlobular and interacinous types of chronic interstitial pancreatitis. Now he went on to demonstrate by the perceptive collection of instances that severe injury to Langerhans' islands is followed by diabetes. A second autopsy provided him with a clue to the cause of some other pancreatic disorders. This time the patient had died of hemorrhagic pancreatitis with fat necrosis, and Opie found a stone blocking the ampulla of Vater. By recourse to animal experiment and diligent postmortem search he demonstrated that the diversion of bile to the pancreas will cause hemorrhagic pancreatitis and that fat necrosis is due to digestion by a ferment liberated from the gland tissue. Studying the pancreas further, reconstructing natural processes with the aid of thought and test, he dispelled much of the mystery that hung about the disorders of the organ and in less than five years had become by right of achievement the authority on pancreatic diseases.

1. As serving the best interests of inaccuracy, this inferential account has been withheld from Dr. Opie. His doings will be dealt with as natural phenomena, which they truly are, and hence as fair game for inferences.

Some one has remarked that once an investigator has entered seriously on his work, his is always a point to point activity, consecutive, never leaping, a gliding progress on the earth of facts. The idea is debatable, though there can be no gainsaying that what a scientist learns in his early, haphazard period is in most instances what he tends to press into service afterward when working toward the unknown.² Opie's early scientific years had seen excursions on a variety of themes besides those just mentioned. Now, however, he had entered on his way. The study of leukocytic enzymes, in which he next engaged, seems to have derived directly out of his experience with the pancreatic ferments. He had just been appointed one of the first group of members of the Rockefeller Institute for Medical Research, at the age of 31, after six years in the department of pathology at Johns Hopkins.

Opie had by this time become a sharply incisive experimenter. No professed biochemist, he taught himself to be a sufficient one for his new purposes. Inducing pleural exudates and removing these at different times, he showed that the polymorphonuclear leukocytes elaborate an enzyme, leukoprotease, which is much like trypsin, whereas the large mononuclear elements coming later into the exudate fluid possess a very different enzyme, lymphoprotease, resembling pepsin. In the blood there are antienzymes capable of neutralizing them both. The interplay of these factors accounts for the breaking down of abscesses, for the resolution they undergo and for numerous other inflammatory phenomena. To pass on to a study of the enzymes of the epithelioid cell, to the conditions which result in "cold abscesses" and to the multifarious complexities of the tuberculous process was to proceed along an opened path. Not a few of the facts Opie discovered at this time, like his disclosure of the significance of the islands of Langerhans, have become the basic commonplaces of modern pathology. They have been rapidly depersonalized, as happens with all facts of large import; yet it is well to recall to whom they are due.

Opie was in the midst of his studies of tuberculosis when in 1910 he was asked to go to St. Louis to help reorganize the medical school of Washington University and become professor of pathology. This happened at a time when he felt that he had been too long away from the autopsy table, when he had come to the belief, as stated to an asso-

2. It can be said of such "command performances" as Humphry Davy's safety lamp and J. S. Haldane's disclosure of the cause of miner's cramps that they are only apparent exceptions to the rule, since these men were exquisitely fitted by reason of knowledge to the tasks they undertook. But what about some of the deeds of Pasteur, and what of such a *tour de force* as Pavlov's study of mental responses after years amidst the vegetative organs?

ciate, that he must continually scrutinize the workings of disease in the human organism if he was to try to understand them. He had lately become interested in how liver necrosis comes about, and had shown, in corollary to the problem, that bacteria and poisons when acting together will produce the hepatic damage which results in atrophic cirrhosis. Thus some insight was provided for the first time into how the disease may come about. Soon after arriving at Washington University he found out how diet might be used to ward off the hepatic and renal effects of alcohol, phosphorus and chloroform, knowledge which has proved applicable in practice. Also he examined into local anaphylactic phenomena in relation to the allergy of tuberculosis and by adroit experiments disclosed the parts played by antigen, antibody and cell, thus reducing this problem to relatively simple terms.

As the years passed at Washington University, years of observation of the diseased human fabric, Opie gradually acquired that wide-ranging knowledge of tuberculous processes which has since been part of his power, and when he was invited in 1923 to be professor of pathology at the University of Pennsylvania and director of the laboratories of the Henry Phipps Institute for the Study, Treatment and Prevention of Tuberculosis, he had fashioned his abilities to precisely such purposes.

Up to this time Opie had been what practitioners call a "laboratory man," even during an interval when he had studied influenza and pneumonia in army camps during the war. But now, though directing a laboratory in which tuberculosis was studied by experimental methods, he turned his personal strength to an examination of the disease in human beings. For he seems to have concluded that nature and the community already had experiments under way which were constantly yielding weighty yet unregarded results. The shift that he made in technic throws a sharp light on his resourcefulness. It is well to look anew at how he found things out. He had always used the simplest of methods and the sharpest of wits. On starting to study clinical tuberculosis in Philadelphia he pressed into use the familiar aids—the postmortem examination, the roentgen ray, the tuberculin reaction, the social service station, the visiting nurse—but he utilized these for clearly delineated ends. Examining public school children who seemed healthy and exploring their family background, matching the patience of the bacillus with his own, he and his co-workers proved, in the course of nine years, that "the spread of tuberculosis occurs in large part by long drawn out family or household epidemics in which the disease is slowly transmitted from one generation to the next." No need to remark to doctors that this is knowledge which saves.

And he found out more. Scrutinizing the bodies of persons who had died of "galloping consumption" in adult life, he noted no signs there of tuberculous infection in childhood, such as might have conferred resistance to the disease. With this knowledge he proceeded to study fulminant tuberculosis in Negroes. And in Jamaica, where the disease is notably prevalent, he disclosed a horrid state of affairs. The Jamaican Negroes flock from the country to the towns to work, and are all innocent of tuberculous infection when they arrive; but it soon reaches them in the hovels where they pass the night. They are readily infected, and once they have become tuberculous they die about three times as fast as do whites. Many of them, going home for their last weeks, bring the pestilence to their families. Nearly the same state of affairs holds true in China, as Opie learned by working at the Peiping Union Medical College.

It will be seen that steadily and unavoidably, through the implications of his discoveries, Opie became a humanitarian, in a telling sense of the word. Nothing has been said thus far of the numerous men who have joined their efforts to his, or of his gifted and generous activities as medical teacher, organizer, adviser and administrator, or of the happy family life which has added to his strength. Nor will more be said here, for this is a story of research, an attempt to tell the history of a mind. The phrase has been used before and in a great connection. Reading Dr. Opie's papers, going along with him from year to year by this means, one sees adventurous thought and imagination, directness, simplicity and a devoutness, which are the man himself. Every one who deals with the phenomena of pathology soon comes to know that nature often speaks her secrets with a still, small voice out of a dense thicket of happenings. He who would hear and comprehend can have no pride of intellect, no fixed preconceptions; he can only listen intently and ask himself what he may have heard. This has been Opie's way in science—and in life. Those who talk with him become aware of more besides wisdom, of a modesty which seems a personal tribute. So indeed it is, though it is the same modesty with which Opie greets a fact.

For any properly zestful scientist there are continually beckoning just over the way the themes to which he might have given himself. Opie has always looked across toward the cancer problem and the kindred diseases leukemia and Hodgkin's disease. Today, freed from administration and teaching, absolved in mind from the duties his own discoveries had laid on him in relation to tuberculosis, he has turned to these themes as specially invited guest of the Rockefeller Institute. And his response to the challenge of the unknown has always been to discover.

This number of the *ARCHIVES* celebrates the man in his work, as has already been said. The scientific papers which follow bespeak achieve-

ments by Dr. Opie which are more personal than any of the shining discoveries he has made; they tell by implication of the admiration and friendship he has called forth. War needs have kept not a few of his former associates from sending in papers, and the rude exigency known as "space limitations" has prevented more. But there are many contributors to the present volume besides those who have written themselves into it. A host of persons, reading, will give thought to Eugene Opie as man or inspiring spirit. Together, readers and writers alike, we find pride in what he is and has done, and we await with eager confidence what he yet will do.

PEYTON ROUS, M.D., New York.

**PUBLICATIONS OF EUGENE L. OPIE
FROM 1898 TO 1941**

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SUPRASELLAR TUMORS RELATED TO THE PARS INTERMEDIA OF THE HYPOPHYSIS

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This article is merely a review of a great number of suprasellar tumors encountered in the neurosurgical service of Dr. W. E. Dandy, who gave me the opportunity to study them. Of course, they are not all alike. While 17 of the tumors were diagnosed as adamantinoma and smaller numbers of tumors were composed of squamous epithelial cells or were of cholesteatomatous character, my interest was concentrated on a very much larger number (113) which in histologic type were practically identical and appeared to correspond with that tissue which in the normal hypophysial structure is best represented by the pars intermedia.

There is an enormous literature devoted to the difficult problem of determining the exact course of the development of that anlage which becomes the hypophysis with its stalk and related structures. The papers of Mihalkovics,¹ Erdheim,² Herring,³ Tilney,⁴ Duffy⁵ and especially those of Atwell⁶ give the results so far established. Essentially there is the upgrowth of an epithelial lobule from the roof of the pharyngeal cavity, at first connected by an open duct, which later closes. The upper part (Rathke's pouch) is thick walled; lateral lobes give rise to the pars intermedia and with the downgrowth of the tubular process from the floor of the third ventricle of the brain which becomes the posterior lobe of the hypophysis, the surrounding extensions of the pars intermedia which form the pars tuberalis are closely related. The remaining portion of Rathke's pouch becomes the anterior lobe, later to show very striking differentiation of three types of cells by their specific granulations.

While this has been worked out in the study of embryos in the course of their development, it is still possible in sections of the hypophysis of the human adult which include the stalk and the base of the brain with the tuber cinereum to distinguish very sharply the pars

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intermedia from the more specialized anterior lobe. Its cells are often in irregular clumps; frequently they form small cysts with a colloid content, and then the lining cells present a more cylindric form with some evidence of cilia on their free surfaces. Often the cells extend in strands or groups into the substance of the posterior lobe and are also to be found scattered along the stalk up to the base of the third ventricle, where they are closely connected with the pars tuberalis, so far as it remains visible.

The neoplasm which occurs in so many cases forms a mass which generally lies above the diaphragm of the sella turcica, although frequently it extends down through it so as to compress the hypophysis and partially surround its stalk. The further growth, generally with a recognizable capsule, projects upward and forward so as to compress the optic chiasm and one or both of the optic nerves; thence it extends into the floor of the third ventricle and upward so as to occupy the cavity of the latter and destroy the fornix or the lateral portions of that level of the brain. The symptoms produced are almost always the same: disturbance of vision with bilateral hemianopia and often complete blindness of one eye, weakness, loss of libido, beginning obesity and other symptoms practically resembling the Fröhlich syndrome, all of which are evidently due to the effects of pressure not only on the hypophysis but on the optic nerves and even the related blood vessels.

Operative removal has involved incision into the capsule of the tumor with curetting of the contents, which are generally soft and brownish. It is difficult to feel sure of the extent of involvement of the hypophysis itself except in those cases in which it has been possible to study this structure at autopsy, and then it is found that the anterior lobe is compressed or surrounded by the tumor cells, which sometimes invade where they are continuous with the remains of the pars intermedia, and surround or push aside the posterior lobe and the stalk. From this it is evident that one cannot say that the tumor actually springs from the thin layer of cells which form the ordinarily recognizable pars intermedia; rather it arises, perhaps, from that same type of cell which is distributed along the stalk or even up into the pars tuberalis.

Microscopically, in this tumor one finds an extraordinary uniformity of structure, best seen in the accompanying photographs. The cells are alike throughout; they tend to be rather cylindric in form around capillaries, and often there are slightly irregular spaces between the strands of such cells. In some cases, however, the strands are broader and made up of several cells closely approximated. The cylindric form of the cells is not always so obvious; in some instances they are irregular or polygonal and clumped together, but in general the blood supply is adequate. None of the great number of stains which have been tried will bring out anything specific in the nature of granules or anything else which would allow one to recognize the cells. This is true of

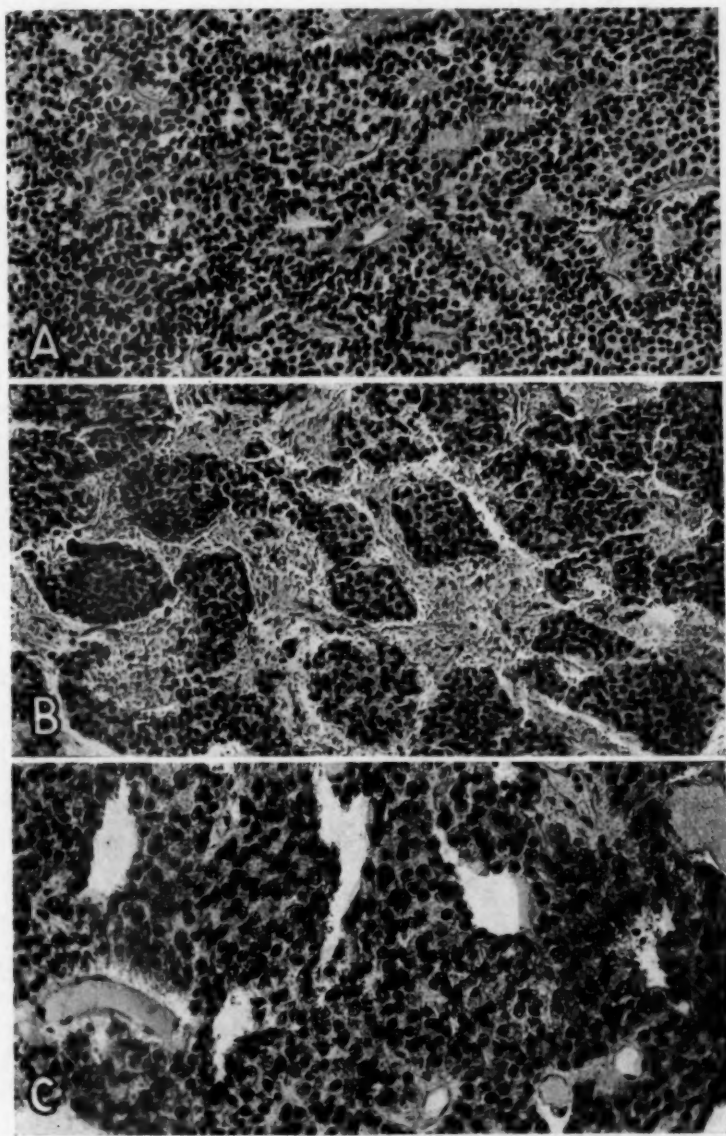


Fig. 1.—Tumors derived from such parts of the craniopharyngeal duct as are destined to form the pars intermedia, or from cells along the hypophysial stalk, including the pars tuberalis.

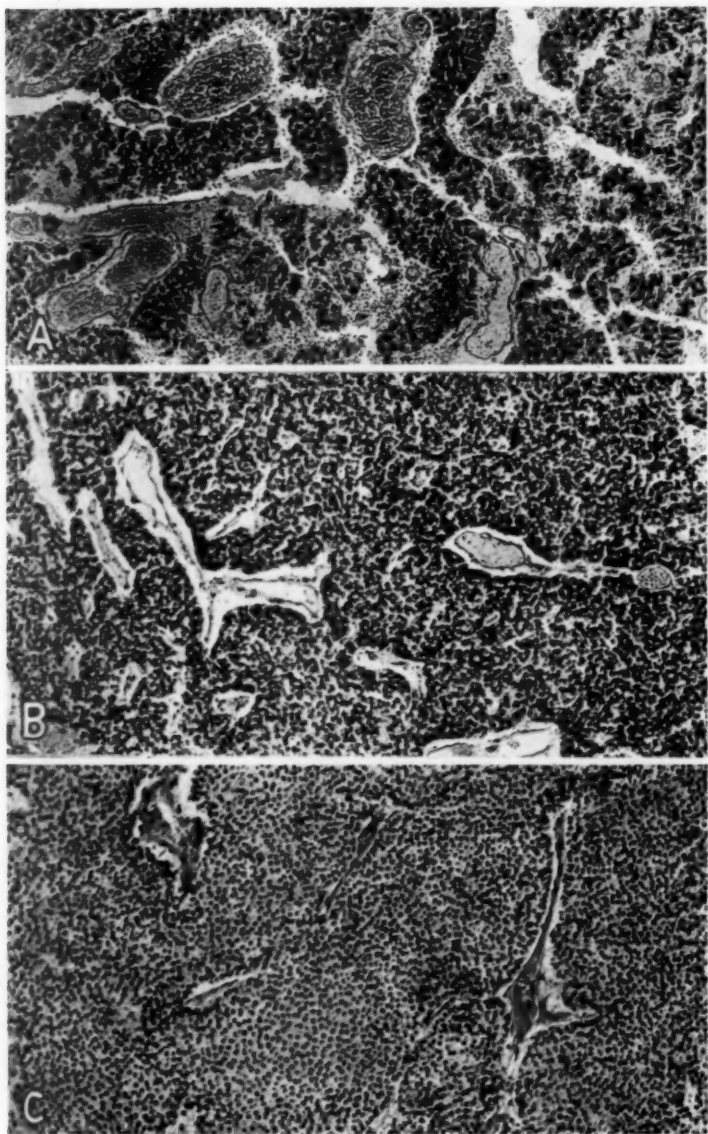


Fig. 2.—Other tumors derived from those parts of the craniopharyngeal duct which evolve into the pars intermedia, or from cells along the hypophyseal stalk, including the pars tuberalis.

the cells of the normal pars intermedia not only in man but in animals, such as the cow or horse, in which the pars intermedia forms a wide, sharply outlined band of tissue.

When the possible source of this tumor is considered, there arises first the habit, so widespread, of speaking of it as chromophobe adenoma of the hypophysis. Of course, in great numbers of cases chromophobe adenoma is encountered at autopsy in the anterior lobe of the hypophysis but that tumor is a spherical nodule, well inside the anterior lobe, somewhat compressing the surrounding granulated cells but seldom extending to the capsule. It is composed of cells rather differently arranged from those of the tumor under consideration and usually spread apart, the cells connected by elongated processes, so that it does not appear comparable with the tumor which grows from outside the anterior lobe.

As to the adenoma composed of eosinophil cells, related to acromegaly, there is no similarity and no discussion is necessary.

A difficulty arises in relation to the Cushing syndrome, which I have always thought to be due to a pars intermedia nodule within the anterior lobe, although this suggestion has been violently contradicted. It was because of the morphologic character of those cells which are cylindric and ranged round capillaries, so unlike the familiar basophil cells, that this idea was put forward.

As to other tumors which arise in positions similar to those described here, there is, of course, adamantinoma, which evidently springs from some displaced embryonic cell group of the type ordinarily developed to furnish enamel to the teeth, but the mechanism of this displacement is not more easily explained than the occurrence sometimes of such a growth in the tibia.

Erdheim has emphasized the occurrence of carcinomatous growth in this situation, and in the present material carcinoma has appeared in 2 or 3 cases. In others squamous epithelial cells have been found in small groups along the stalk and capsule of the hypophysis. Up to the present cholesteatoma has not appeared in this series. There is at least a variety of possibilities in the course of such a complex developmental process as that of the hypophysis with its infundibular stalk for the origin of very different types of tumor.

The aim of the present paper is to emphasize the great frequency of the occurrence of a type of tumor almost always exactly identical in microscopic structure which seems to originate from those cells which normally go to form the pars intermedia with its extensions along the stalk to the pars tuberalis.

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INFLUENCE OF VARIOUS TYPES OF IMMUNIZATION
ON THE GENESIS OF EXPERIMENTAL HEMO-
LYTIC STREPTOCOCCUS ARTHRITIS

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Studies on the production of experimental arthritis in relation to sensitization and immunization with products of bacteria have been relatively few. Herry¹ in 1914 showed that if a strain of diplococci was ground and the extract obtained injected into the knee joint of a rabbit, and eight to fifteen days later an intravenous injection of the living organisms was given, arthritis usually developed in the prepared knee joint. Faber² in 1915 sensitized joints by intra-articular injections of killed green streptococci and stated that when the same organisms were subsequently injected intravenously they lodged more readily in the sensitized joints. He regards this sensitization as specific within set limits, although he did not claim that the sensitization was specific for various strains of streptococci. Swift and Boots³ in 1923 sensitized joints of rabbits by intra-articular injection of killed nonhemolytic streptococci and subsequently infected the animals by intravenous injection of a homologous streptococcus culture. Because arthritis developed in many nonsensitized as well as in sensitized joints, they concluded that sensitization was not an important factor in the localization of bacteria in joints. Bieling⁴ expressed the belief that the arthritis developing in horses during immunization with repeated intravenous injections of hemolytic streptococci was not due directly to metastatic infection but was modified considerably by the initial injections of heat-killed organisms.

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The studies of Klinge⁵ indicate that in rabbits sensitized by intra-articular injections of horse serum inflammatory lesions of the joints and heart develop that bear some similarity to the histologic picture usually associated with rheumatic fever. Bruun⁶ was able to confirm Klinge's observations in part, but certain experiments could not be duplicated. Magrassi⁷ and more recently Collins and Goldie⁸ reported experiments indicating that hypersensitivity may be of importance in modifying the arthritis produced by various micro-organisms.

Since the reported experimental studies had varied so greatly both in method and in results obtained, it seemed desirable to do further experiments of this nature to determine what effect immunization or sensitization would have on the development of streptococcic arthritis in rabbits.

The following experiments were planned to determine (1) the effect of general immunization and sensitization and (2) the effect of intra-articular sensitization on the development of arthritis in rabbits.

METHOD

Normal male and female albino rabbits weighing from 2,000 to 2,400 Gm. were used.

Cultures.—Eighteen hour broth cultures of *Streptococcus haemolyticus* (strain AB 13) were used for the preparation of the formaldehydized vaccine, the nucleoprotein and the filtrates.⁹ The same strain was used for the production of arthritis. A vaccine prepared from a culture of hemolytic *Staphylococcus aureus* obtained from a furuncle was also used for intra-articular injection.

Immunization.—The hair over the side and back was first removed with an electric clipper, and each animal was given four courses of six intradermal injections (0.1 or 1.0 cc. amounts) of hemolytic streptococci that had been killed with formaldehyde in a final concentration of 0.08 per cent. The animals were permitted to rest for one week after each course. In the majority there developed a high degree of cutaneous sensitivity to vaccine, filtrate or streptococcic nucleoprotein.

A comparable, but somewhat larger, number of animals was given similar intravenous injections simultaneously. The same degree of cutaneous sensitivity did not develop, and the skin tests made with vaccine or streptococcic nucleoprotein produced a nodular reaction associated with slight erythema and edema.

Diagnosis of Arthritis.—The animals were observed daily for clinical arthritis, which was readily detected by observation and palpation. The most important signs were (1) the animal's favoring an extremity, (2) a limp and (3) localized swelling of a joint.

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EXPERIMENTS ON INTRACUTANEOUSLY AND INTRAVENOUSLY
IMMUNIZED RABBITS

Seventeen rabbits were each given repeated intravenous injections of 0.1 cc. of a formaldehydized hemolytic streptococcus culture over a period of eight weeks, so that each received a total of 2.4 cc. Ten other rabbits received the same amount of vaccine intracutaneously. One week after immunization, these animals, together with 24 controls, were each given 2.0 cc. of a living broth culture of *Str. haemolyticus* (table 1). Arthritis developed in 10 of the 17 (58.8 per cent) intravenously immunized animals. Three of the intracutaneously immunized animals, with a high degree of cutaneous sensitivity, died within forty-eight hours after injection of the culture. However, arthritis developed in 4 of the 7 (57.1 per cent) surviving animals and in 16 of the 24

TABLE 1.—Incidence of Arthritis Produced with Intravenous Injections of Living Hemolytic Streptococcus Cultures in Normal and in Intravenously or Intracutaneously Immunized Rabbits

Rabbit Group	Route of Immunization	Amount of Vaccine, Cc.	Amount of Culture, Cc.	Rabbits	Rabbits with Arthritis	
					Number	Per Cent
I	Intravenous.....	2.4	2.0	17	10	58.8
	Intracutaneous.....	2.4	2.0	7	4	57.1
	Control.....	...	2.0	24	16	66.6
II	Intravenous.....	24.0	0.1	16	8	50.0
	Intracutaneous.....	24.0	0.1	16	1	6.3
	Control.....	0.1	19	2	10.5
III	Intravenous.....	24.0	0.01	28	7	25.0
	Intracutaneous.....	24.0	0.01	28	2	7.1
	Control.....	0.01	31	2	6.5

(66.6 per cent) controls. This experiment showed that there was no conspicuous difference in the incidence of arthritis in the control, the intravenously immunized or the intracutaneously immunized group.

The animals in group II were immunized with somewhat larger amounts of vaccine, so that over a period of eight weeks each of the 16 intravenously and 16 intracutaneously immunized animals received a total of 24.0 cc., or ten times the amount given to those of group I. Shortly after immunization, these animals, together with 19 controls, were given intravenously 0.1 cc. of a living hemolytic streptococcus culture. The incidence of arthritis in this series of animals was less than that in group I; however the disease developed in 8 of 16 intravenously immunized animals, whereas it appeared in only 1 intracutaneously immunized and 2 control rabbits.

Because the smaller amount of culture, namely, 0.1 cc., produced arthritis in such a high percentage (50 per cent) of the intravenously immunized animals in group I, it seemed desirable to determine the

effect of a still smaller dose. Accordingly, 0.01 cc. of culture was given to a group of 87 rabbits, comprised of 28 intravenously and 28 intracutaneously immunized animals and 31 controls. In 7 (25 per cent) of the intravenously immunized animals arthritis developed whereas only 2 of the intracutaneously immunized and control groups had any involvement of joints.

We tried to correlate the arthritis that developed in these animals with the degree of cutaneous sensitivity at the time of the infection and also with the level of circulating antibodies, namely, agglutinins and anti-group-specific precipitins. No correlation, however, could be demonstrated.

Arthritis frequently appeared earlier in the intravenously immunized than in the control animals. In 8 of 25 it was observed forty-eight hours after the intravenous injection of living culture. In nonimmunized animals it did not appear before the third day; usually it was noted about the fifth or sixth day. The incidence of arthritis in the intracutaneously immunized animals was so low that no conclusion could be drawn as to the time of onset. It is of interest that arthritis developed in 1 such animal within twenty-four hours after the intravenous injection of living streptococcus culture.

The synovial fluid and tissue of one or more of the involved joints of each animal were cultured. Streptococci were usually recovered for a considerably longer period from the synovial tissue than from the synovial fluid. Since the three groups were killed at different times following the onset of arthritis, and because the incidence of arthritis varied in each group, it was difficult to evaluate the significance of the cultural data. As far as we could determine, streptococci were recovered from the three groups with equal facility and for about the same length of time after infection.

It is of considerable interest that the intravenously immunized animals with arthritis had an average of only one joint in each animal involved, whereas in comparable control animals the average number of affected joints was about two per animal. We also observed that the joints of intravenously immunized animals were characterized by less swelling and less loss of mobility than those of the control animals. The number of intracutaneously immunized animals in which arthritis developed was too small to warrant comparison with the other groups.

Histologic sections of the synovial membrane from at least one joint of every animal with arthritis were prepared. The microscopic picture was similar to that previously described.¹⁰ The inflammatory exudate was characterized by an accumulation of neutrophils throughout the synovial membrane and in the synovial fluid. Usually within forty-eight hours monocytes and lymphocytes were present in fairly large numbers.

10. Cecil, R. L.; Angevine, D. M., and Rothbard, S.: *Am. J. M. Sc.* **198**: 463, 1939.

In some instances the inflammation subsided and the synovial membrane assumed its normal appearance. The usual course was for the inflammatory exudate to become chronic and persist for several months; in such instances the predominant picture was one of mononuclear leukocytes and lymphocytes in the subsynovial tissue. Frequently these cells were grouped together as foci, and they somewhat resembled lymph follicles. Similar collections of such cells, associated with fibrosis, were frequently seen in the epiphysal bone marrow. After the chronic inflammatory process had persisted for several months, a pannus of fibrous connective tissue usually covered areas of cartilage that showed some degree of degeneration.

There was no conspicuous difference in either the type or the extent of the inflammatory process in the three groups. It must be borne in mind, however, that in this experiment most of the animals were killed at a time when the acute inflammatory reaction had subsided. To determine the character of the inflammatory exudate in the different groups, it would be necessary to study the synovial membranes from a considerable number of animals from each group at the onset of the disease.

This experiment shows that it was possible to produce arthritis in rabbits previously immunized intravenously with an amount of living hemolytic streptococcus culture that seldom produced arthritis in normal animals. The explanation of these observations must depend to some extent on speculation, as we did not attempt to determine the state of sensitivity of the synovia of any of the animals following immunization. It seems probable, however, that during the period of immunization with formaldehydized cultures of *Str. haemolyticus* considerably larger quantities of the products of the streptococcus reached the joints of intravenously immunized than of intracutaneously immunized animals. So far as the latter group is concerned, it is probable that as cutaneous sensitization developed, increasing amounts of antigen remained fixed at the sites of injection and relatively small amounts reached the joints.

EXPERIMENTS WITH INTRA-ARTICULAR INJECTIONS OF HEAT-KILLED HEMOLYTIC STREPTOCOCCUS CULTURE OR NUCLEOPROTEIN

The following experiments were undertaken to determine the effect of intra-articular injections of heat-killed hemolytic streptococcus culture or streptococcic nucleoprotein on the localization in the same joint of intravenously injected living culture.

The hair was clipped over both knee joints and the skin sterilized with 70 per cent alcohol. The injected material was given in 0.5 cc. volume through a no. 26 needle. To facilitate intra-articular injection, the leg was flexed on the thigh, thereby producing slight tension on the joint capsule. The material was injected near the medial margin of the patella. The joint was then observed for evidence of inflammation. In some instances the periarticular swelling increased slightly for a short time after the first or subsequent injections. In the majority of

instances no change was noted; however, in a few cases the rabbit held the knee in a flexed position for several days after such an injection.

EXPERIMENT 1.—Each of 20 rabbits received four injections of 0.5 cc. of a 1:50 dilution of formaldehyde-killed hemolytic streptococcus culture into the right knee joint at four day intervals. After the last injection, they were allowed to rest for ten days, at which time twelve were given intravenously 0.01 cc. of living streptococcus culture. The remaining 8 were not given intravenous injections but were kept as controls (table 2).

Arthritis developed in only 1 animal. Those in which arthritis did not develop after the first intravenous injection were given additional injections of increasing amounts (0.1, 1.0, 2.0 and 4.0 cc.) of streptococcus culture at ten day intervals.

The intra-articular injections of vaccine occasionally produced slight swelling, but this rapidly subsided. In some instances there was a tendency to favor the hindleg; however, this sign varied considerably from day to day. Because of this, the clinical diagnosis of arthritis in a joint that had received an intra-articular injection was less accurate than that on a joint that had received no previous treatment.

Clinical arthritis developed in joints other than the knees in 7 of 12 animals. The amount of inflammation produced by the intra-articular injections alone was relatively slight (fig. 1*A* and *B*). However, in animals that received intra-articular injections and then intravenous injections, there was an extensive inflammatory process, characterized by an infiltration of mononuclear leukocytes and lymphocytes (fig. 2*A* and *B*). Hemolytic streptococci were recovered from the synovial tissues of only 2 of the 12 rabbits. The microscopic examination of the synovial membranes frequently revealed extensive chronic inflammation when there was no clinical and little gross evidence of synovitis. Because of this, it seemed appropriate to consider the degree of inflammation in the synovial membrane as the most suitable index with which to compare the different joints. The extent of the inflammation is recorded as from 1 to 4 plus.

It is evident that the inflammation in the synovia from the right knee joints of animals that received intravenous injections of living culture was much greater than that in the controls (13 to 20, table 2). Of the former group, all had extensive synovitis ranging from 2 to 4 plus, whereas in the controls the inflammation was considerably less and was recorded as from 1 to 2 plus; in 2 animals there was no demonstrable inflammation.

It is evident from this experiment that when formaldehydized hemolytic streptococci were injected repeatedly into the knee joint sufficient sensitization was produced so that subsequent intravenous injections of living hemolytic streptococcus culture caused an inflammatory reaction in the synovial membrane. Although there was a difference in the inflammation in either knee joint, it should be noted that arthritis developed about as frequently in the nonsensitized as in the sensitized joints.

EXPERIMENT 2.—Because of the reported success¹ in localizing streptococci in joints following intra-articular injection of ground bacteria, it seemed desirable to determine the effect of streptococcic nucleoprotein when injected intra-articularly.

It was first necessary to determine the amount of nucleoprotein that would produce a minimal amount of synovitis with as few injections as possible. It was

also important to determine the most appropriate interval of time between the intra-articular and the intravenous injections. The agglutinin titers of the synovial membranes of both the prepared and the nonprepared joints were determined after the last intra-articular injection. The serum agglutinins were determined at the same time. The various injections, agglutinin titers and degrees of inflammation

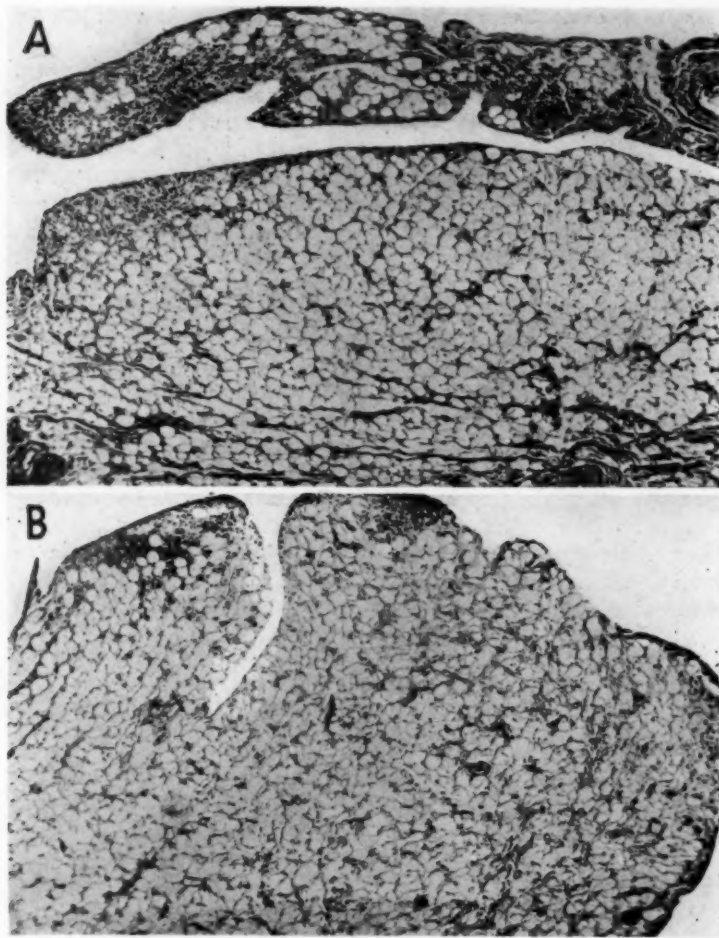


Fig. 1.—*A* (rabbit 14, table 2), synovial membrane with a synovial villus; $\times 50$. There is a slight degree of chronic inflammation. The section was taken through the patellar region of the right knee. The rabbit had received four intra-articular injections of 0.5 cc. of formaldehydized hemolytic streptococcus culture diluted 1:50, at four day intervals, and was killed one month after the last injection.

B (rabbit 15, table 2), synovial membrane; $\times 50$. The animal was treated in the same manner as rabbit 14 in *A*.

of the synovial membranes are recorded in table 3. It is evident that there is considerable variation in the titers of different animals after similar injections and

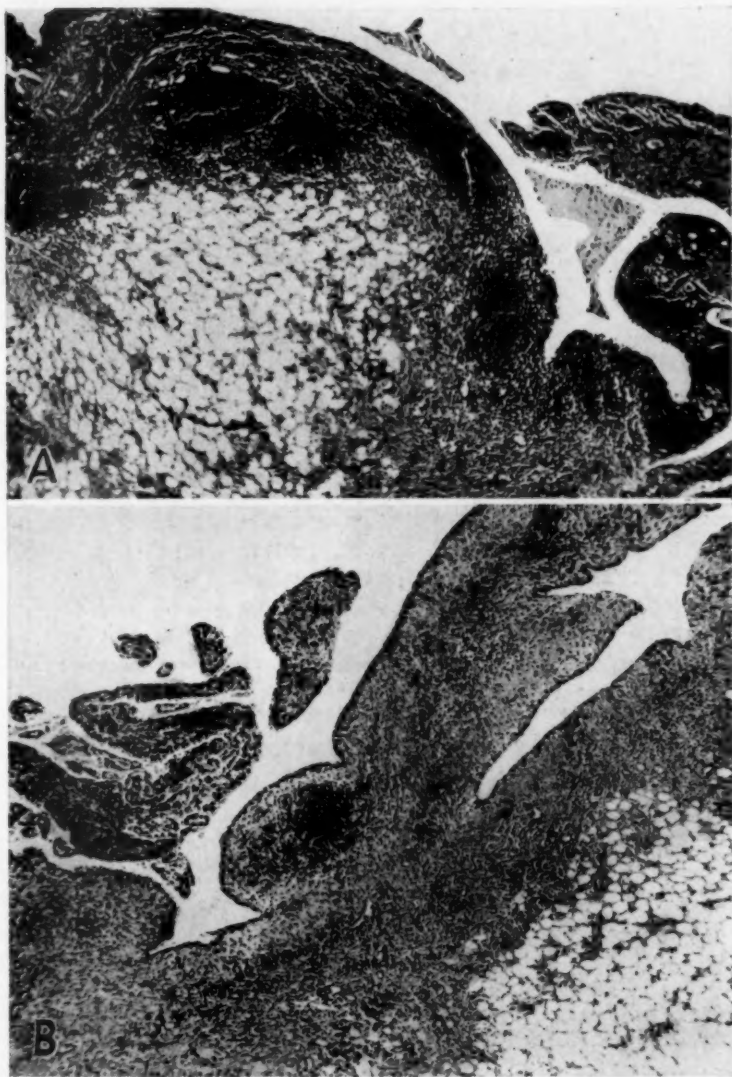


Fig. 2.—*A* (rabbit 12, table 2), extensive lymphocytic infiltration with follicle formation; $\times 50$. The right knee joint was prepared in the same way as those of rabbits 14 and 15 in figure 1; on the twentieth day thereafter 0.01 cc. of living hemolytic streptococcus culture was given intravenously, and on the twenty-sixth day the rabbit was killed.

B (rabbit 6, table 2), lymphocytic infiltration with beginning fibrosis of sub-synovial tissue; $\times 50$. The right knee joint of this animal was treated in the same manner as those of rabbits 12, 14 and 15. Thereafter the animal received four intravenous injections of streptococcus culture (0.01, 0.1, 1.0 and 2.0 cc. on the twentieth, thirty-third, forty-first and forty-ninth days) and was killed on the sixty-second day.

TABLE 2.—Degree of Inflammation in the Synovial Membrane of the Right Knee Joint After Four Intra-Articular Injections of Formaldehyde-Killed Streptococci (0.5 Cc. of a Culture Diluted 1: 50) Followed by Intravenous Injections of Hemolytic Streptococcus Culture (Amounts of from 0.01 Cc. to 2.0 Cc.)

Rabbit	Intravenous Injections of Culture	Degree of Inflammation in Right Knee	Other Joints with Arthritis
1.....	5	++++	
2.....	5	++++	
3.....	5	+++	
4.....	5	+++	
5.....	5	++	Right elbow
6.....	4	++++	Left elbow
7.....	4	++++	
8.....	2	++++	Right shoulder
9.....	2	+++	Right shoulder
10.....	2	+++	Left wrist
11.....	2	++	Right elbow
12.....	1	++++	Right wrist
13.....	0	++	
14.....	0	++	
15.....	0	+	
16.....	0	+	
17.....	0	+	
18.....	0	+	
19.....	0	0	
20.....	0	0	

TABLE 3.—Degree of Synovial Inflammation Produced by Varying Numbers of Intra-Articular Injections and with Different Amounts of Streptococcic Nucleoprotein

Rabbit	Amount of Dilute Nucleo-protein, Cc.	Injec-tions	Interval After Last Injection, Weeks	Agglutinin Titer		Degree of Inflammation
				Serum	Synovial Membrane	
1:2 dilution						
1	0.5	1	2	+
2	0.5	1	2	0	0	±
3	0.5	2	2	1:40	1:20	+
4	0.5	2	2	0	0	++
5	0.5	2	2	++
6	0.5	2	3	1:40	1:40	0
7	0.5	2	3	1:160	0	+
8	0.5	2	3	0	0	0
9	0.5	2	3	0	0	+
10	0.5	3	2	0	1:1,280	++
11	0.5	3	2	1:640	1:2,560	+++
12	0.5	3	3	0	1:40	±
13	0.5	3	3	0	0	0
1:10 dilution						
14	0.5	2	2	0	1:80	+
15	0.5	2	2	1:80	0	+
16	0.5	2	2	0	0	+
17	0.5	2	2	0	0	+
18	0.5	2	3	1:160	0	0
19	0.5	2	3	0	1:40	±
20	0.5	2	3	0	0	0
21	0.5	2	3	0	1:40	±
22	0.5	2	3	0	1:40	±
23	0.5	2	3	1:80	1:160	0
24	0.5	2	3	0	0	±
25	0.5	2	3	0	0	0
26	0.5	2	3	1:20	0	0
27	0.5	2	3	1:40	0	±

that a definite titer could not be anticipated after any given number of intra-articular injections. In only 4 of 15 rabbits with agglutinins in either the blood serum or the synovial tissue were they present in both.

Streptococcic nucleoprotein diluted 1:10 when injected twice into the knee joints of 10 rabbits at a four day interval produced inflammation of such a degree that after three weeks there was a minimal amount of inflammation with only slight proliferation of the synovial cells (table 3). Accordingly, this amount was selected for the subsequent experiment.

TABLE 4.—*Extent of Inflammation in the Synovial Membranes of 26 Rabbits That Received Two Intra-Articular Injections of 0.5 Cc. of Dilute Streptococcic Nucleoprotein (1:10). After an Interval of Three Weeks, 15 of Them Received a Single Intravenous Injection of Living Hemolytic Streptococcus Culture*

Rabbit	Amount of Culture Injected Intravenously, Cc.	Right Knee		Left Knee	
		Degree of Inflammation	Foci of Lymphocytes	Degree of Inflammation	Foci of Lymphocytes
1.....	1.0	++++	++	0	0
2.....	1.0	++++	+	0	0
3.....	1.0	++++	+	0	0
4.....	1.0	+++	+	0	0
5.....	1.0	+++	+	0	0
6.....	1.0	+++	+	0	0
7.....	1.0	+++	+	0	0
8.....	1.0	+++	+	0	0
9.....	1.0	++	+	0	0
10.....	1.0	++	+	0	0
11.....	1.0	++	+	0	0
12.....	1.0	++	0	0	0
13.....	1.0	++	0	0	0
14.....	1.0	++	0	0	0
15.....	1.0	++	0	++	0
16 ¹	++	+		
17.....	...	+	0		
18.....	...	+	0		
19.....	...	+	0		
20.....	...	+	0		
21.....	...	0	0		
22.....	...	0	0		
23.....	...	0	0		
24.....	...	0	0		
25.....	...	0	0		
26.....	...	0	0		

Each of 26 rabbits received two intra-articular injections of 0.5 cc. of streptococcic nucleoprotein diluted 1:10. After three weeks, 15 of them were given intravenously 1.0 cc. of living hemolytic streptococcus culture, and all were killed after from one to seven days. The joints were examined grossly, the synovial membrane cultured and sections made for histologic study.

The results are given in table 4. The inflammatory reaction in the group that received intra-articular injections was more extensive than that in the controls. Lymphocytes accumulated as foci in 11 of the 15 animals. One usually does not see such accumulations of lymphocytes so soon after the onset of arthritis. This observation is of considerable

importance because the usual acute inflammation seen in the synovial membrane is apparently modified in some manner by the sensitizing injection, so that during the first week, instead of the usual acute synovitis, there is a more chronic inflammatory process, characterized by lymphocytes and plasma cells accumulated as foci, especially in the synovial villi. It is also of considerable interest that streptococci were recovered with difficulty from the synovia of the animals that received intra-articular injections.

EXPERIMENT 3.—The work of Swift and Boots⁸ suggested that intra-articular injection of heat-killed streptococci will not sensitize a joint sufficiently to localize specifically different strains of streptococci. Nevertheless, it seemed desirable to determine whether bacteria might lodge more frequently in joints prepared with streptococcic nucleoprotein than in those treated with a foreign protein, such as horse serum.

Twenty-one rabbits were used (table 5). Each received from one to three intra-articular injections of streptococcic nucleoprotein diluted 1:2 or horse serum diluted 1:32. In these dilutions, the total protein contents of nucleoprotein and horse serum were approximately the same. The nucleoprotein was injected into the right and the horse serum into the left knee joint. Two weeks after the last intra-articular injection, 13 rabbits were given either one or two intravenous injections of hemolytic streptococcus culture and killed after from one to twelve days. It can be seen that there was only a slight amount of inflammation in either knee joint of the control animals, whereas in those receiving the intravenous injections of culture there was considerable inflammation in the synovial membrane. Two rabbits (2 and 9, table 5) that received intra-articular injections were given a single intravenous injection of horse serum and killed three days later. There was no inflammatory reaction in either knee joint. The absence of reaction in the serum-treated joints suggests either that the quantity of horse serum injected intra-articularly was not sufficient to sensitize the joint or that the provocative intravenous injection was too small. We have had no difficulty eliciting such a reaction in the synovial membrane when larger amounts of horse serum were given intra-articularly.

The results obtained on this relatively small group of rabbits indicate that streptococcic nucleoprotein produced sufficient sensitization in the synovial membrane so that subsequent intravenous injections of hemolytic streptococci produced an inflammatory reaction. Such a reaction was not elicited in the contralateral joint that received horse serum. No experiments were made to determine whether strains other than the one employed would elicit such a reaction.

A few observations carried out with a formaldehydized vaccine prepared from a culture of *Staph. aureus*, although not conclusive, are of sufficient interest to mention.

Each of 12 rabbits received four intra-articular injections into both knees; 0.5 cc. of formaldehydized hemolytic streptococcus culture diluted 1:50 was injected into the right and a similar amount of *Staph. aureus* vaccine into the left knee joint. After eight days the rabbits received varying doses of living streptococcus culture intravenously. The animals were killed when arthritis developed or

when they had received five injections of culture. The periods of observations varied from six to fifty days.

In this experiment, clinical evidence of arthritis was not noted more frequently in the knees than in other joints. However, in eleven of the twelve animals the inflammatory process in the synovial membranes of joints that received killed streptococci was more pronounced than that in joints that received killed staphylococci. The inflammation

TABLE 5.—*Degree of Inflammation of the Synovia in Rabbits That First Received One, Two or Three Intra-Articular Injections of 0.5 Cc. of Dilute Streptococcic Nucleoprotein (1:2) into the Right and 0.5 Cc. of Dilute Horse Serum (1:32) into the Left Knee Joint. After Two Weeks, Most of the Rabbits Received One or More Intravenous Injections of Hemolytic Streptococcus Culture*

Rabbit	Intra-Articular Injections	Intravenous Injections	Interval Between Last Intra-Articular Injection and Death, Days	Degree of Inflammation	
				Right Knee	Left Knee
1	1	14	0	0
2	1	Horse serum, 1.0 cc.....	17	0	0
3	1	Hemolytic streptococci, 1.0 cc.....	17	+++	+
4	1	Hemolytic streptococci, 1.0 cc.....	19	++	0
5	1	Hemolytic streptococci, 1.0 cc.....	18	+	0
6	1	Hemolytic streptococci, 1.0 cc.....	14	0	0
7	2	Horse serum, 2.0 cc.....	19	++	0
8	2	21	+	0
9	2	21	0	0
10	2	Hemolytic streptococci, 1.0 cc.....	18	+++	0
11	2	Hemolytic streptococci, 1.0 cc.....	20	+++	0
12	2	Hemolytic streptococci, 1.0 cc.....	14	++	+
13	2	Hemolytic streptococci, 1.0 cc.....	17	+	0
14	3	21	+	+
15	3	21	+	0
16	3	21	0	0
17	3	Hemolytic streptococci, 0.1 cc.....	21	+++	0
18	3	Hemolytic streptococci, 0.1 cc., 1.0 cc..	22	+++	0
19	3	Hemolytic streptococci, 0.1 cc.....	23	+++	0
20	3	Hemolytic streptococci, 0.1 cc.....	26	+++	0
21	3	Hemolytic streptococci, 0.1 cc.....	24	++	++ (arthritis)

in the staphylococcus-treated joints was more extensive than that in controls that received intra-articular injections of staphylococcus vaccine alone. This is presumably due to the similar common antigen that is present in gram-positive organisms.

SUMMARY

Arthritis developed more frequently in animals immunized with repeated intravenous injections of formaldehydized hemolytic streptococci than in either intracutaneously immunized or control rabbits.

Relatively small amounts of hemolytic streptococcus culture were required to produce arthritis in the intravenously immunized group.

Intra-articular injections of heat-killed hemolytic streptococci or streptococcic nucleoprotein altered the synovial tissue of the knee joints of rabbits so that subsequent intravenous injections of living streptococcus culture elicited a local inflammatory reaction in the synovial membrane of the prepared joints.

In animals that first received intra-articular injections, the inflammatory exudate in the synovial membrane was chronic and characterized by lymphocytic foci, in contrast to the more acute synovitis usually developing in joints with no previous intra-articular injections. It was more difficult to recover the organism from treated than from non-treated joints.

Intravenous injections of living hemolytic streptococcus culture elicited a more extensive inflammatory reaction in the synovial membrane of joints treated with intra-articular injections of streptococcus vaccine or nucleoprotein than in the joints prepared with either staphylococcus vaccine or horse serum.

These experiments indicate that the products of hemolytic streptococci injected either intravenously or intracutaneously into rabbits reached the synovial membrane and presumably sensitized it. In animals given such treatment, small amounts of homologous culture injected intravenously were capable of eliciting an inflammatory reaction in the synovial membrane more frequently than in control animals. The inflammatory process was of a more chronic nature than that usually observed in nonsensitized animals.

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RELATIONSHIP OF COCCIDIOIDOMYCOSIS TO CALCIFIED PULMONARY NODULES

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The occurrence of roentgenologically demonstrable pulmonary nodules having the density of calcium in those who fail to react to tuberculin is of interest because of the question of etiology.

Nelson, Mitchell and Brown¹ observed calcified pulmonary nodules in 27 of 62 children residing in an institution in Ohio, who failed to react to tuberculin. They concluded that low grade or healed tuberculous lesions may be associated with failure to react to tuberculin. In Indiana, Crimm and Short² observed calcification in 191 patients who failed to react to tuberculin. They assumed that the calcification was of tuberculous origin and that these patients were anergic. In Philadelphia, Hetherington, McPhedran, Landis and Opie³ observed pulmonary nodules in 8.5 per cent of 82 children who showed negative reactions to tuberculin, while 3.7 per cent of this group were considered as having tuberculosis of the tracheobronchial nodes.

In a carefully controlled study in Williamson County, Tenn., Gass, Gauld, Harrison, Stewart and Williams⁴ observed calcified pulmonary nodules in 48 and 34 per cent of 563 white and 143 Negro children, respectively. The incidence of calcified nodules among the white children was not significantly different as between those who had negative and those who had positive reactions to tuberculin, while among the Negro children calcification was less frequent in the group who did not react to tuberculin.

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From the Office of Indian Affairs, Department of the Interior, Washington, D. C., and the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa.

1. Nelson, W. E.; Mitchell, A. G., and Brown, E. W.: *Am. Rev. Tuberc.* **37**: 311, 1938.

2. Crimm, P. D., and Short, D. M.: *Am. Rev. Tuberc.* **39**:64, 1939.

3. Hetherington, H. W.; McPhedran, F. M.; Landis, H. R. M., and Opie, E. L.: *Am. Rev. Tuberc.* **20**:421, 1929.

4. Gass, R. S.; Gauld, R. L.; Harrison, E. F.; Stewart, N. E., and Williams, W. E.: *Am. Rev. Tuberc.* **38**:441, 1938.

The failure to react to tuberculin in these cases cannot be attributed to the use of tuberculin of low potency. One of us (J. D. A.) has compared samples of the preparation of old tuberculin used by Gass and his co-workers with other preparations of tuberculin, used for the purpose of standardization. The samples of old tuberculin in question were approximately as potent as a sample of old tuberculin prepared by the Staatsinstitut für experimentelle Therapie, Frankfurt on the Main, Germany, and old tuberculin prepared by the United States Bureau of Animal Industry and were definitely more potent than the international standard old tuberculin.

A sample of the old tuberculin used by Gass and his co-workers was also compared at the Henry Phipps Institute with purified protein derivative tuberculin. With the first dose, both tuberculins gave positive reactions in 20 of 50 persons; with the second dose, 15 of 30 persons reacted to purified protein derivative and 17 to old tuberculin.⁴ Dahlstrom⁵ found that of 2,490 persons who reacted to tuberculin 256 subsequently had reversible reactions. In the latter group calcified pulmonary nodules were found in 11, active childhood tuberculosis in 2 and a condition in 5 that was diagnosed as latent tuberculosis or suspected of being this.

In the course of studies dealing with tuberculosis among some of the Indians of the United States and Alaska, roentgenologic examinations were made of the chests of 3,024 children and young adults ranging in age from 1 to 19 years who failed to react to the intracutaneous injection of 0.00002 and 0.005 mg. of purified protein derivative tuberculin. With but few exceptions these subjects were examined roentgenologically either shortly before or shortly after they were tested with tuberculin and were subsequently roentgenologically examined annually.

The same sample of a purified protein derivative tuberculin prepared for us by Dr. Florence Seibert was used throughout these studies. Fresh dilutions were made daily from the concentrated 1 per cent stock solution. This tuberculin was highly specific, as indicated by the fact that 93.3 per cent of those vaccinated with BCG one year previously reacted to the injection of either 0.00002 or 0.005 mg. of this preparation. The potency of the sample used was compared with a sample of a preparation of old tuberculin which we had used extensively in the past. Of 704 children tested simultaneously with 0.00002 mg. of tuberculin and 0.01 mg. of old tuberculin, 372 reacted to both tuberculins; 315 failed to react to either sample, while 2 gave doubtful reactions. In 2 instances a positive reaction was obtained with purified protein derivative but not with old tuberculin, while in 6 instances the reaction to old tuberculin was positive but that to purified protein derivative tuberculin was negative. In 5 instances a doubtful reaction

5. Dahlstrom, A. W.: *Am. Rev. Tuberc.* **42**:471, 1940.

to purified protein derivative was observed but a definite one to old tuberculin, and in 2 instances the reaction to purified protein derivative was definite but that to old tuberculin was doubtful.

The roentgenograms were interpreted by one of us (R. M. S.) without any previous knowledge of the tuberculin reactions. All lesions of a doubtful nature were reviewed by different members of the staff of the Henry Phipps Institute.

When the results of the roentgenologic examinations were analyzed, it was found that among 704 children living on the Pima Agency, near Phoenix, Ariz., who did not react to tuberculin, calcified pulmonary nodules were observed in 102, or 14.5 per cent. Among 419 Shoshone and Arapaho children living on the Wind River Agency, Wyo., 1 only of those who failed to react to tuberculin was observed to have a

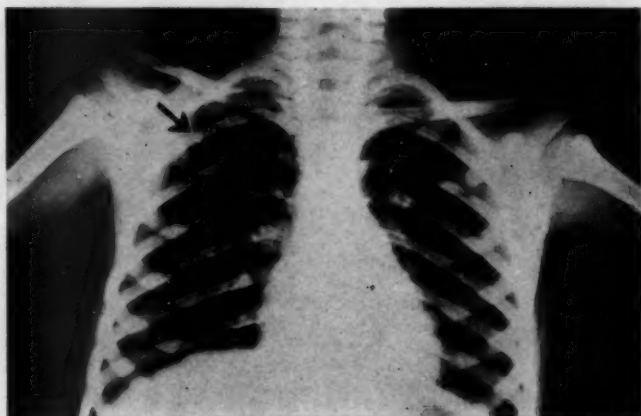


Fig. 1.—Calcified nodule in the upper lobe of the right lung of a 3 year old child who gave no reaction to 0.005 mg. of tuberculin but gave a 3 plus reaction to 1:1,000 dilution of coccidioidin.

calcified pulmonary nodule, while among 332 Chippewa children living on the Turtle Mountain Agency who did not react to tuberculin, 15, or 4.5 per cent, had calcified pulmonary nodules. Among the 592 Sioux children of the Rosebud Agency and the 977 Indian children living in Southeastern Alaska who failed to react to tuberculin, 6, or 1.0 per cent, and 2, or 0.2 per cent, respectively, presented calcified pulmonary nodules.

Because of the high incidence of calcified pulmonary nodules in "tuberculin-negative" children living on the Pima Agency, Ariz., further studies were undertaken.

The character of the calcified pulmonary nodules occurring in the persons on the Pima Agency who did not react to tuberculin was noteworthy. These nodules appeared as sharply defined dense rounded or irregular shadows, ranging in size on the roentgen film from 3 to 8

mm. at their widest point (fig. 1). In 94 cases there was but one calcified nodule; in 7 instances there were two and in 1 instance four. They occurred on the right side in 45 instances, on the left side in 49 and on both sides in 8. In approximately 50 per cent of the cases the calcified nodules occurred in the middle third of the lungs, in 30 per cent in the upper third and in 20 per cent in the lower third. They were observed most frequently at the periphery of the lung, occasionally within the shadow of the heart, but rarely at the hilus.

The age distribution of the 102 persons showing calcified pulmonary nodules is presented in table 1.

In view of the failure of 102 persons with calcified pulmonary nodules to react to tuberculin, the possibility that these calcified nodules were of a nontuberculous nature was considered, and because of the occurrence of pulmonary coccidioidal granuloma in the neighboring state of California, coccidioidomycosis was investigated as a possible etiologic factor.

TABLE 1.—*Calcified Pulmonary Nodules in Persons Residing on the Pima Agency, Ariz., Who Did Not React to Tuberculin*

Age in 5 Year Periods	Persons Nonreactive to Tuberculin	Persons with Calcified Pulmonary Nodules	
		Number	Per Cent
5 to 9.....	375	44	11.7
10 to 14.....	272	49	18.0
15 to 19.....	57	9	15.8
Total.....	704	102	14.5

The occurrence of pulmonary lesions caused by *Coccidioides immitis* has been known since the initial recognition of this disease in North America by Rixford in 1893.⁶ Coccidioidomycosis and tuberculosis in the clinical manifestations and pathologic aspects present striking similarities. Dickson⁷ stated that pulmonary coccidioidal granuloma is not infrequently mistaken for tuberculosis, and Jacobson⁸ concluded that the differentiation of the two diseases must depend on laboratory findings. Carter⁹ stated that the roentgenologic findings in cases of coccidioidal granuloma may consist of enlargement of the hilar and mediastinal nodes, pulmonary infiltration, abscess formation, consolidation or pleural exudation. Calcification of the hilar nodes was observed, and calcification in the lung parenchyma resembled the classic Ghon tubercle.

6. Rixford, E.: *Occidental M. Times* 8:326 and 704, 1894.

7. Dickson, E. C.: *Tr. A. Am. Physicians* 44:284, 1929.

8. Jacobson, H. P.: *Fungous Diseases*, Springfield, Ill., Charles C. Thomas, Publisher, 1932, pp. 239 and 206.

9. Carter, R. A.: *Radiology* 26:551, 1936.

INVESTIGATIVE PROCEDURE

Since the studies of Kessel,¹⁰ Dickson¹¹ and Smith¹² have indicated that the reaction to coccidioidin has definite value in diagnosing coccidioides infection and determining its spread, this test was used on the children of school age living on the Pima Agency, in Arizona.

The coccidioidin used throughout these studies was obtained from Dr. Charles E. Smith, of Leland Stanford University. It was prepared by growing several strains of *C. immitis* on a synthetic liquid culture medium consisting of asparagine, dipotassium phosphate, sodium citrate, magnesium sulfate, ferric citrate, dextrose, glycerin and water, removing the organism by filtration through a bacteria-proof filter and preserving the unheated filtrate with a 1:10,000 dilution of merthiolate.

To determine the specificity of the coccidioidin reaction and to ascertain its relation, if any, to the tuberculin reaction, simultaneous intracutaneous injections of purified protein derivative tuberculin and coccidioidin were made. Dilutions of both preparations were prepared daily with sterile physiologic solution of sodium chloride. New glassware and syringes, each marked distinctly and conspicuously, for each preparation were used in preparing the dilutions and making the injections. The initial intracutaneous injection consisted of 0.1 cc. of a 1:1,000 dilution of coccidioidin and of 0.00002 mg. of tuberculin. If no reactions were observed at the sites of injection forty-eight hours later, 0.1 cc. of a 1:10 dilution of coccidioidin and 0.005 mg. of tuberculin were again injected. This procedure was subsequently modified to the making of a single injection of 0.1 cc. of a 1:100 dilution of coccidioidin. The reactions were interpreted and graded according to the method proposed by Aronson.¹³

RESULTS AND OBSERVATIONS

Since a large percentage of the children living on the Pima Agency were found to react to coccidioidin, the investigation was extended to other areas in order to ascertain whether the high incidence noted at this agency was significant.

The results of the coccidioidin and tuberculin tests made in various parts of the United States and in southeastern Alaska are presented in table 2.

It will be observed in table 2 that a large percentage of children of school age living on the Pima, San Carlos and Sells agencies and a small percentage of those living on the Fort Apache Agency reacted to the injection of 0.1 cc. of a 1:1,000 or to 1:100 dilution of coccidioidin, while in other areas of the United States and Alaska but few reacted to these amounts or to the injection of a 1:10 dilution of coccidioidin. Of the children living on the Fort Apache Agency who reacted to coccidioidin, a number had attended school at the Pima Agency for several years, while others had visited or lived on the San Carlos Agency.

10. Kessel, J. F.: *Am. J. Trop. Med.* **19**:199, 1939.

11. Dickson, E. C.: *Am. Rev. Tuberc.* **38**:722, 1938.

12. Smith, C. E.: *Am. J. Pub. Health* **30**:600, 1940.

13. Aronson, J. D.: *Am. Rev. Tuberc.* **30**:727, 1934.

It is evident from table 2 that there is no relation between the coccidioidin and the tuberculin reaction. Furthermore, the incidence of the coccidioidin reaction, unlike that of the tuberculin reaction, shows no appreciable increase after the age period of 5 to 9 years. When the incidence of the coccidioidin reaction among those of preschool age living on the Pima Agency was analyzed at yearly intervals, it was found that of 33 children less than 1 year of age 2, or 6.1 per cent,

TABLE 2.—Comparison of Results of Coccidioidin and Tuberculin Tests in Some Areas of the United States and Alaska

Location		Under 5 Years		5 to 9 Years		10 to 14 Years		15 to 19 Years		20+ Years		Total— All Ages	
		No. Tested	Per Cent Positive	No. Tested	Per Cent Positive	No. Tested	Per Cent Positive	No. Tested	Per Cent Positive	No. Tested	Per Cent Positive	No. Tested	Per Cent Positive
Pima Agency, Ariz.	C	181	32.0	550	86.7	492	94.1	192	96.4	4	100.0	1,419	83.6
	T	181	7.2	522	22.4	360	56.7	172	72.6	4	75.0	1,239	37.2
San Carlos Agency, Ariz.	C	83	57.8	198	95.4	232	97.8	113	97.8	15	100.0	641	92.0
	T	83	6.0	198	26.7	232	51.7	113	76.1	15	100.0	641	43.5
Sells Agency, Ariz.	C	6	66.6	156	93.7	133	97.0	90	96.7	31	83.9	418	94.2
	T	6	0.0	157	38.2	132	65.2	87	81.6	29	89.5	411	59.1
Fort Apache Agency, Ariz.	C	0	...	189	8.9	220	11.8	118	27.1	16	37.5	543	14.9
	T	0	...	189	67.7	220	88.6	118	95.7	16	93.1	543	89.8
Rosebud Agency, S. D.	C	0	...	55	1.8*	63	0.0	11	0.0	0	...	129	0.7*
	T	0	...	55	58.3	63	69.9	11	100.0	0	...	129	67.4
Turtle Mountain Agency, N. D.	C	0	...	46	0.0	46	0.0	7	0.0	0	...	99	0.0
	T	0	...	46	50.0	46	60.8	7	85.0	0	...	99	58.7
Wind River Agency, Wyo.	C	0	...	78	0.0	59	0.0	22	0.0	0	...	159	0.0
	T	0	...	78	61.5	59	70.0	22	100.0	0	...	159	72.9
Philadelphia	C	0	...	0	...	0	...	0	...	117	5.9*	117	5.9
	T	0	...	0	...	0	...	0	...	117	97.4	117	97.4
Southeastern Alaska	C	0	...	9	0.0	38	0.0	209	1.0*	29	3.4*	285	1.1*
	T	0	...	9	100.0	38	94.7	209	94.5	29	96.5	285	96.1

* = percentage positive to 0.1 cc. of a 1:10 dilution of coccidioidin.

C = percentage positive to 0.1 cc. of a 1:1,000, a 1:100 or a 1:10 dilution of coccidioidin.

T = percentage positive to 0.0002 and 0.006 mg. of purified protein derivative.

reacted. Among 36 children 1 to 2 years of age 1, or 2.8 per cent, reacted, while of the 38 ranging in age from 2 to 3 years of age 17, or 44.7 per cent, reacted. Of the 40 children 3 to 4 years old 14, or 35 per cent, reacted to coccidioidin, while among the 35 children 4 to 5 years of age 20, or 57.1 per cent, reacted. It is evident, therefore, that infection with *C. immitis* is infrequent in children in the first two years of life and that it increases markedly after the age of 2 years. It is significant that this sharp rise occurs at an age when children are more apt to be walking and playing with soil.

In its gross appearance the inflammatory reaction induced by the injection of coccidioidin resembled that induced by tuberculin, although

usually the induration was less marked. The edema and the swelling reached a maximum in about forty-eight hours, after which time they gradually subsided. In the more severe reactions the induration persisted for several weeks and was frequently followed by desquamation and pigmentation at the site of injection. Up to the present time no involvement of the regional lymphatics or lymph nodes has been observed.

Dickson¹¹ recommended that a maximum dose of 0.1 cc. of a 1:10 dilution of coccidioidin be used. We have observed, however, that among tuberculous patients residing in Philadelphia who gave no history of having lived or visited the West, 5.9 per cent of 117 adults reacted to the injection of 0.1 cc. of a 1:10 dilution of one lot of coccidioidin, while with another lot of coccidioidin 6.3 per cent of 135 tuberculous patients reacted. These results suggest that the reaction induced by the larger dose of coccidioidin may be nonspecific, and we recommend that for routine surveys the maximum dose of coccidioidin of known potency be 0.1 cc. of a 1:100 dilution.

That the reaction to coccidioidin bears no relation to sensitivity to tuberculin or to sensitivity to other bacterial proteins may be deduced from the fact that in Alaska 285 Indians tested simultaneously with 0.00002 mg. of purified protein derivative tuberculin and 0.1 cc. of a 1:1000 dilution of coccidioidin showed 96 per cent reactions to tuberculin, with severe reactions occurring in many, and no reactions to coccidioidin in 1:1,000 dilution and only 3 reactions to 0.1 cc. of a 1:10 dilution of coccidioidin. On the other hand, the occurrence of heteroallergy in Alaska is evident since, of 95 Indians tested simultaneously with 0.00002 mg. of purified protein derivative tuberculin prepared from human type tubercle bacilli and with 0.00002 mg. of purified protein derivative tuberculin made from avian tubercle bacilli, 96 per cent reacted to the tuberculin from human type bacilli while 50 per cent reacted to the tuberculin from avian tubercle bacilli. Similarly, of 141 Indians in Alaska, 85 per cent reacted to 0.00002 mg. of tuberculin while, of the same group, 66 per cent reacted to 0.00002 mg. of protein prepared from *Brucella abortus*.¹⁴ The reactions to the avian tuberculin and to the protein from *Br. abortus* occurred in natives of southeastern Alaska who do not ordinarily consume milk or poultry; nor were cattle or fowl found in the villages where these tests were made. Among the Sioux Indians of Rosebud, S. D., who failed to react to coccidioidin, definite reactions were observed in 36 per cent of those tested with 0.00002 mg. of trichloroacetic acid

14. Otero, P. M., and Gonzalez, L. M.: *Proc. Soc. Exper. Biol. & Med.* 38:703, 1938.

precipitate tuberculin from *Mycobacterium smegmatis*, in 36 per cent of those tested with the same amount of trichloroacetic acid precipitate tuberculin from *Mycobacterium ranae*, in 17 per cent of those tested with trichloroacetic acid precipitate tuberculin from *Mycobacterium marinum*, 0.4 per cent of those tested with that from *Mycobacterium phlei* and in 67 per cent of those tested with purified protein derivative tuberculin from human type tubercle bacilli.

The trichloroacetic acid precipitate tuberculin used in these studies was supplied by Mr. H. J. Henderson, of the Henry Phipps Institute, who made the different preparations by growing the cultures on Long's synthetic medium, concentrating the unheated material by ultrafiltration and precipitating the protein with trichloroacetic acid.¹⁵

That the intensity of reactions to coccidioidin is more or less directly related to the incidence of coccidioidomycosis is indicated in table 3.

It will be observed there that the 3 plus reactions occurred more frequently among those living on the Pima, San Carlos and Sells agencies than among those living at the Fort Apache Agency, where coccidioidomycosis was less frequent and where its presence was due, in all probability, to the return to this agency of persons who attended school or who visited on the Pima and San Carlos agencies.

To study the specificity of the coccidioidin reaction further, intracutaneous injections were made of preparations made in the same manner as coccidioidin, which were furnished to us by Dr. Charles E. Smith, of Stanford University. These preparations were made from cultures of *Histoplasma capsulatum*, *Microsporon canis*, *Blastomyces*, *Sporotrichum* and *Aspergillus niger*. Of those who failed to react or who reacted to coccidioidin, none reacted to the intracutaneous injection of 0.1 cc. of a 1:100 dilution of preparations made from *Blastomyces*, *Sporotrichum* or *A. niger*. Slight but definite reactions were observed in 32 of 42 persons who reacted to coccidioidin and who were tested with 0.1 cc. of a 1:100 dilution of *M. canis*, while among 28 persons who failed to react to coccidioidin none reacted to this preparation. Eleven who reacted to coccidioidin also reacted to the preparation made from *H. capsulatum*, while 2 who failed to react to coccidioidin gave positive reactions to 0.1 cc. of a 1:100 dilution of the histoplasmin. Because of these results an additional sample of histoplasmin was obtained from Dr. C. W. Emmons, of the National Institute of Health. With this preparation no reactions were observed in those who reacted or failed to react to coccidioidin.

15. Henderson, H. J.: *Internat. J. Leprosy* 5:267, 1937.

TABLE 3.—*Degree of Reaction According to Dose of Coccidioidin*

Location	0.1 Cc. of a 1:1,000 Dilution of Coccidioidin						0.1 Cc. of a 1:100 Dilution of Coccidioidin						0.1 Cc. of a 1:10 Dilution of Coccidioidin					
	Positive Degree of Reaction						Positive Degree of Reaction						Positive Degree of Reaction					
	No. Tested	No.	Per Cent	1+	2+	3+	No. Tested	No.	Per Cent	1+	2+	3+	No. Tested	No.	Per Cent	1+	2+	3+
Pima Agency, Ariz.....	1,430	902	63.1	36	62	12	50	42	84.0	2.0	43	50	457	297	60.9	2.0	44	54
San Carlos Agency, Ariz.....	298	161	77.4	13	58	29	521	500	97.7	3.0	45	52	0	0	Not given	injection		
Sells Agency, Ariz.....	231	180	81.8	14	71	15	214	180	88.3	7.0	43	45	0	0	Not given	injection		
Fort Apache Agency, Ariz.....	106	6	5.6	50	50	0	431	79	18.3	30.0	61	19	82	1	1.2	0	0	100

1+ means that the diameter of the edema ranged from 6 to 10 mm. inclusive; 2+, from 11 to 20 mm. inclusive; 3+, above 20 mm.

OCCURRENCE OF CALCIFIED PULMONARY NODULES

Our original observations on the Pima Agency indicated the occurrence of calcified pulmonary nodules in 14.5 per cent of those who failed to react to tuberculin. The occurrence of such lesions in cases in which the tuberculin test gave negative results was investigated in other parts of the United States and Alaska and was correlated with the frequency of the coccidioidin reaction in these areas. The results are summarized in table 4.

It is evident from table 4 that calcified pulmonary nodules occurred in a significant number of those who failed to react to tuberculin and who live in areas where reactions to coccidioidin were observed in the vast majority of the population. On the other hand, an insignificant number of calcified nodules was observed in those who failed to react to tuberculin and who live in areas where the reaction to coccidioidin is infrequent or absent. It is noteworthy that the incidence of calcified pulmonary nodules did not increase with increase in age as one might have expected if the underlying lesion was of tuberculous origin.

The failure to observe calcified pulmonary nodules in cases in which the tuberculin test gave negative results in areas free of coccidioidomycosis is not to be attributed to absence of calcifications in these areas. Thus among the people with positive tuberculin reactions at Fort Apache calcified pulmonary nodules were observed in 11.3 per cent of those examined roentgenologically, while among the Shoshone and Arapaho Indians of the Wind River Agency, Wyo., 16 and 25 per cent, respectively, showed calcified pulmonary nodules. Among the Sioux Indians of Rosebud, S. D., 14 per cent of the general population had calcified pulmonary nodules, while among the Indians of southeastern Alaska such lesions were observed in 11 per cent of the general population. The figures for the occurrence of calcified pulmonary nodules in those who reacted only to tuberculin and those who reacted only to coccidioidin, as well as the figures for the occurrence of such nodules in those who reacted and those who failed to react to both tuberculin and coccidioidin were compared, and the results are presented in table 5.

It will be observed from table 5 that when the numbers studied were statistically significant the percentage with calcified pulmonary nodules among those who reacted to both tuberculin and coccidioidin was appreciably higher than the sum of the percentages with such nodules among those who reacted only to tuberculin or only to coccidioidin. This suggests that calcification is more apt to occur when both tuberculosis and coccidioides infection occur in the same person than when either of these infections occurs singly.

From the data already presented it is evident that in the Pima, San Carlos and Sells agencies located in Arizona there is a high incidence

TABLE 4.—Incidence of Calcified Pulmonary Nodules in Persons with Negative Reactions to Tuberculin Correlated with Incidence of Coccidioidin Reaction

Location	Under 5 Years			5 to 9 Years			10 to 14 Years			15 to 19 Years			Total—All Ages			Coccidioidin Reaction	
	No. with Negative Reaction to Tu-berculin	Calcified Nodule No.	Per Cent	No. with Negative Reaction to Tu-berculin	Calcified Nodule No.	Per Cent	No. with Negative Reaction to Tu-berculin	Calcified Nodule No.	Per Cent	No. with Negative Reaction to Tu-berculin	Calcified Nodule No.	Per Cent	No. with Negative Reaction to Tu-berculin	Calcified Nodule No.	Per Cent	No. Tested	Per Cent with Coccidioidin Reaction
Pima Agency, Ariz.....	0	0	...	375	44	11.7	272	40	18.0	57	0	15.8	704	103	14.5	1,419	83.6
San Carlos Agency, Ariz.....	0	0	...	121	23	19.0	112	17	15.2	27	0	200	40	15.4	641	92.0
Fort Apache Agency, Ariz.....	0	0	...	61	0	25	1	4.0	1	0	87	1	1.1	543	14.9
Wind River Agency, Wyo.....	187	0	...	147	1	0.7	73	0	...	12	0	419	1	0.24	159	0.0
Turtle Mountain Agency, N. D.....	79	1	1.3	103	8	4.8	80	6	7.5	5	0	332	15	4.5	90	0.0
Rosebud Agency, S. D.....	74	0	...	277	3	1.1	163	3	1.6	48	0	592	0	1.0	129	0.7*
Southeastern Alaska.....	559	1	0.2	328	1	0.3	121	0	...	19	0	977	2	0.2	285	1.1*

* This was the percentage positive to 0.1 cc. of a 1:10 dilution of coccidioidin.

TABLE 5.—*Relation of Tuberculin Reaction and of Coccidioidin Reaction to Occurrence of Calcified Pulmonary Nodules*

Group	Flona Agency			San Carlos Agency			Fort Apache Agency			Total—All Agencies		
	No. from Roentgen- ograms Were Taken	Calcified Pulmonary Nodules	Per Cent	No. from Roentgen- ograms Were Taken	Calcified Pulmonary Nodules	Per Cent	No. from Roentgen- ograms Were Taken	Calcified Pulmonary Nodules	Per Cent	No. from Roentgen- ograms Were Taken	Calcified Pulmonary Nodules	Per Cent
Those who reacted to tuberculin but not to coccidioidin	18	2	11.1	5	1	20.0	325	49	15.1	348	52	14.9
Those who reacted to coccidioidin but not to tuberculin	251	37	14.7	294	39	13.3	1	0	546	78	13.9
Those who reacted to both tuberculin and coccidioidin	318	69	21.7	235	74	31.1	66	14	21.2	619	157	25.3
Those who failed to react to both tu- berculin and coccidioidin	30	1	3.3	5	1	20.0	64	1	1.6	99	3	3.0
Total	617	109	17.7	539	115	21.3	455	64	14.0	1,612	288	17.9

of reactions to coccidioidin, while calcified pulmonary nodules were roentgenologically demonstrated in 13.5 per cent of 281 and 13.4 per cent of 299 children of school age living on the Pima and San Carlos agencies, respectively, who were not reactive to tuberculin. On the other hand, on the Fort Apache Agency the incidence of coccidioidin reactions is low, while calcified pulmonary nodules were observed only in 1.6 per cent of the 65 subjects who were negative to tuberculin. That the character of the soil and climatic conditions may have a bearing on the presence of *C. immitis* is suggested by Dickson,¹⁶ who expressed the belief that infection occurs most frequently in arid regions where the climate is hot and dry. It is recognized that coccidioidomycosis occurs most frequently among those whose occupations bring them into frequent contact with the soil, such as well drillers, linemen, fruit and cotton pickers. While repeated attempts to culture *C. immitis* from the soil have given negative results, Stewart and Meyer¹⁷ succeeded in isolating the organism from specimens of earth collected in Kern County, Calif., and Smith¹⁸ and co-workers succeeded in isolating the organism from the soil in other areas of California. Mycologic studies of the soil of the several agencies in Arizona under investigation are being conducted by Dr. C. W. Emmons, of the United States Public Health Service, to determine whether there is any relation between the occurrence of immitis in the soil and the incidence of the coccidioidin reaction.

The marked differences observed in the incidence of the coccidioidin reaction on the Pima, Sells and San Carlos agencies, on the one hand, and the infrequency with which this reaction and calcification were observed in the Fort Apache Agency, on the other hand, made it desirable to assemble data describing the physical characteristics of the terrain and soil, the type of the prevailing flora, the climatic conditions and the amount of precipitation. The geographic relation of the different agencies included in this study is presented in figure 2.

The Pima Agency, situated in the southwestern part of Arizona approximately 40 miles (64 kilometers) southeast of Phoenix, Ariz., has a population of 4,841 Pima Indians, living on 465,196 acres (188,262 hectares). The reservation, which has an average elevation of 1,800 feet (548.5 meters), is divided by the Gila River into two approximately equal parts. It is traversed by United States highway 87, which runs from east to west, and by the Southern Pacific Railroad.

The reservation consists of large areas of flat plains, which are broken by several barren low mountain ranges. A number of irrigation ditches traverse the reservation in different directions. The light-colored soil is low in humus and is composed of an alluvial deposit of sandy loam and caliche, which is highly calcareous and

16. Dickson, E. C.: Arch. Int. Med. **59**:1029, 1937.

17. Stewart, R. A., and Meyer, K. F.: Proc. Soc. Exper. Biol. & Med. **29**: 937, 1932.

18. Smith, C. E.: Personal communication to the authors.

alkaline, consisting of the carbonates of calcium and magnesium. The climate is arid or semiarid, and temperatures of 100 F. or higher occur from April to November, while the nights are usually cool. The sun shines on an average of 84 per cent of the time annually. During the colder months frosts are rare and the humidity is low. The precipitation over an extended period of years has averaged 9.8 inches (24.5 cm.) per year. The precipitation occurs chiefly from July to October and during the winter months. Wind velocity averages 5.8 miles (9.28 kilometers) per hour, although dust storms are not uncommon.

The homes in most instances are one story structures of adobe brick or of adobe plastered on willows. The floor consists of packed earth, while the roof is made of willows or brush covered by a layer of dirt. The houses contain from two to four rooms and are usually overcrowded. Sewage is disposed of on the surface. The Indians on this reservation maintain themselves by farming, cotton picking



Fig. 2.—Relative location of the Indian reservations in the southern part of Arizona.

and cattle raising. The chief crops are alfalfa, cotton and grain, which are grown in the irrigated areas, while cattle are grazed and fattened on the reservation. There are no perennial grasses, but many varieties of cactuses and opuntia abound. Different varieties of mesquite, ironwood, paloverde, creosote bush, greasewood and ocotillo are common, while in the river bottoms marsh tamarisk, cottonwood and willows are found.

The Sells Agency, situated in the west central part of Arizona, is bounded on the south by the Republic of Mexico. It has a population of 5,726 Papago Indians living on 2,773,854 acres (1,122,564 hectares). The elevation ranges in most areas from 1,400 to 2,600 feet (426.5 to 792.5 meters), while the peak of the Baboquivari Mountains reaches 7,600 feet (2,316.5 meters) above sea level. The reservation is located on the edge of the most arid section of the United States and consists of large broad alluvial plains interrupted by small rough barren mountains which arise abruptly out of the desert. It is traversed by a main road, which runs from Tucson to Ajo, Ariz.

The soil is comparable to that found on the Pima Agency. The climate is arid or semiarid and dry, and the days of sunshine average annually 85 to 89 per cent. The humidity is low. Precipitation varies markedly from torrential rains to extreme droughts, its annual precipitation being greatest during the period from July to September and during the winter months. The amount of precipitation on the reservation varies from 5 inches (12.5 cm.) in the southwestern part to 18 inches (45.5 cm.) in the Baboquivari Mountains. The annual rainfall over a period of fifty-three years averages 11.5 inches (29 cm.). Snowfall is rare, and frosts are of short duration. The wind velocity ranges from 5 to 6 miles (8 to 9.5 kilometers) per hour. The temperature range is comparable with that observed on the Pima Agency. There is no irrigation on the reservation, and small subsistence gardens are maintained by some of the Indians. Crops are not raised to any extent. Cattle are grazed over the reservation. The Papago Indians maintain themselves by cattle raising, cotton picking and farm labor in adjacent areas. The vegetation on this reservation is Sonoran in type and resembles that found on the Pima Agency.

The San Carlos Agency is located in the southeastern part of Arizona. It extends over 1,619,717 acres (655,499 hectares) and is populated by 3,116 Apache Indians. It is traversed by United States Highway 60, which passes through the western boundary of the reservation, by United States Highway 70, along the southern border, and by a branch of the Southern Pacific Railroad. On the north the reservation is separated from the Fort Apache Agency by the Salt River, which flows through a deep canyon. The reservation consists of a series of plateaus at levels of 2,000 to 8,000 feet (609.5 to 2,438.5 meters) separated by deep canyons and crowned by narrow mountain ranges. There are two principal soil belts, the desert alluvial belt, comparable to that found on the Pima and Sells agencies, and the brown podsollic soil, which is acid in reaction and rich in humus.

In the southern part the summers are long and hot. Precipitation has ranged from 10 to 14 inches (25.5 to 35.5 cm.) per year in the semiarid southern part to 14 to 20 inches (35.5 to 50.5 cm.) per year in the northern part of the reservation. Fifty-three per cent of the precipitation occurs during the summer months and 25 per cent during the winter months. The Indians live in communities along the Gila and San Carlos rivers, in the southern part of the agency, where the soil is of an alluvial nature comparable to that found on the Pima and Sells agencies. The raising of cattle is the principal source of income, while small plots are used for subsistence gardens. During the summer months many of the Indians migrate to the northern, more mountainous parts of the reservation, where they graze their cattle. The homes in most instances consist of a wikiup, which is a conical habitation measuring 9 to 10 feet (2.5 to 3 meters) in height, made of poles covered by brush, grass and odd pieces of canvas. The central opening at the top permits the escape of smoke from the central fire pit, while the door faces the west. In the southern part of the reservation there is an abundance of creosote bushes, while in the bottom lands willows, mesquite, saltbrush and desert shrubs abound. In the north there is considerable table land with yellow pine forest and grassy parks.

The Fort Apache Agency is located in the eastern part of Arizona and is separated, on its southern boundary, from the San Carlos Agency by a deep canyon, through which flows the Salt River. It extends over 1,664,873 acres (673,677 hectares) and has a population of 2,934 Apache Indians. It is traversed by United States Highway 60, which runs from north to south, and by a branch of the Santa Fe Railroad, which extends to the lumber mill town of McNary in

the north. The reservation consists of a series of plateaus ranging in elevation from 2,900 feet (884 meters) at the canyon of the Salt River to 11,000 feet (3,353 meters) at Baldy Peak in the northwestern corner. A number of deep canyons and streams divide the reservation. The soil is of the brown or reddish brown podsol type, rich in humus and acid or neutral in reaction. The climate is subtropical and the soil semiarid in places. The average temperature is 54 F., which compares with 66 F. in the Pima Agency and 64 F. in the San Carlos Agency. The average annual rainfall is 18 inches (45.5 cm.) per year, while the average annual snowfall is also 18 inches. Frosts are frequent and persistent, while dust storms are infrequent. The homes are of the same type of wikiup used by the Apache Indians of the San Carlos Agency. Vast areas of timber land with frequent large grassy parks of fescue and perennial grasses are present. The trees consist mostly of fir, spruce, yellow pine and oak. Along the streams sycamore and poplar trees are common. The Indians live in communities or camps scattered over the reservation. The main source of income is from the raising of cattle, while small truck gardens provide subsistence.

COMMENT

It has generally been assumed that calcified pulmonary nodules are the resultant of a tuberculous process. While this is undoubtedly the most frequent cause of calcification, due consideration must be given to other factors which may produce degeneration and death of tissue cells, especially in those locations where the injured tissue cannot be evacuated or absorbed.

Frimann-Dahl and Waaler¹⁹ found that roentgenologic shadows having the density associated with calcium could be produced by calcified emboli and thrombi, intra-alveolar ossification, anthracotic and silicotic nodules, calcified nodules or plaques in thickened pleura and deposits of calcium on wood or cellulose. Cox and Smith²⁰ observed calcified pulmonary nodules due to coccidioidomycosis in 4 of 3,000 autopsies in San Francisco. Similar calcified lesions could be produced in both guinea pigs and rats by injections of the spherules of *C. immitis*.

It is generally believed that the failure to react to tuberculin in those showing calcified pulmonary nodules or other lesions assumed to be tuberculous may be due either (1) to a loss of allergy associated with the disappearance of viable tubercle bacilli or (2) to the occurrence of tuberculosis in anergic persons.

With regard to the loss of tuberculin allergy Long²¹ concluded that sensitivity to tuberculin may be due to recent and repeated intake of tubercle bacilli rather than to persistence of allergy induced by the original infection. Dahlstrom⁵ observed a definite relation between the intensity of the tuberculin reaction, the degree of exposure to tuberculous infection and the loss of sensitivity to tuberculin. Loss of allergy was most frequent in the decennium 5 to 15 years. Of 11

19. Frimann-Dahl, J., and Waaler, G.: *Acta radiol.*, 1936, supp. 33, p. 1.

20. Cox, A. J., and Smith, C. E.: *Arch. Path.* 27:717, 1939.

21. Long, E. R.: *Illinois Health Messenger* 12:1, 1940.

persons with calcified pulmonary nodules in whom the tuberculin reaction had reverted from positive to negative, 4 were members of nontuberculous households and 4 members of tuberculous households where the sputum showed no bacilli. Despite these observations, it is not clear why a tuberculous process sufficiently extensive to result in calcification should produce transitory allergy while in the vast majority of instances of persistent and well marked allergy no lesions should be roentgenologically demonstrable during life and at autopsy, in many instances, with respect to both man and animal, no gross or microscopic lesions of tuberculosis are detectable.

With reference to the second explanation, the occurrence of tuberculosis in anergic persons, it is desirable to define "anergy" in its relation to tuberculosis. We suggest that the term "anergy" be applied to the state of those who fail to react to tuberculin of known potency after they have been definitely exposed for a sufficient length of time to viable virulent tubercle bacilli or who fail to react to tuberculin after parenteral administration of a dose of viable, avirulent or killed tubercle bacilli capable of inducing allergy in the vast majority of instances. That the failure to react to tuberculin in a large number of our subjects showing calcified pulmonary nodules cannot be attributed to anergy is proved by the fact that 56 of 62 such persons reacted to purified protein derivative tuberculin one year after the intracutaneous injection of 0.15 mg. of BCG vaccine and among the 40 unvaccinated ones 16 reacted to tuberculin in from one to four years after their initial negative reaction. Confirmation of the absence of allergy in the subjects in question was indicated by the fact that in no instance was there observed either a Koch phenomenon or an accelerated local reaction at the site of injection of the BCG vaccine.

The specificity of the coccidioidin reaction is indicated by the development of sensitivity following infection with *C. immitis*,¹¹ the high incidence of reactions among those living in areas where the disease is endemic and the absence of reactions among those living in areas where the disease has not been reported. The coccidioidin reaction bears no relation to the tuberculin reaction. In both Alaska and South Dakota, where those tested with coccidioidin failed to react, marked sensitivity to tuberculin was noted, while at Fort Apache 14.9 per cent reacted to coccidioidin and 83.8 per cent to tuberculin. Similarly, no reaction to coccidioidin was observed in areas where heteroallergic reactions to proteins from other acid-fast bacteria and from *Br. abortus* were noted.

The presence of characteristic roentgenologically demonstrable calcified pulmonary nodules in persons showing negative as well as in those showing positive reactions to tuberculin in areas where a high incidence of reactions to coccidioidin has been observed suggests that cocci-

doidomycosis may be an etiologic factor. That calcified pulmonary nodules may be due to other factors than tuberculosis and possibly coccidioidomycosis is suggested by the fact that in North Dakota among 15 persons with calcified nodules 10 who were tested failed to react to both tuberculin and coccidioidin.

SUMMARY

The occurrence of roentgenologically demonstrable calcified pulmonary nodules in those who failed to react to tuberculin varied widely in different parts of the United States and Alaska.

Among Indians living on the Pima, San Carlos and Sells agencies, in the arid region of southern Arizona, a large percentage reacted to intracutaneous injections of coccidioidin, while among those examined roentgenologically on the Pima and San Carlos agencies a high incidence of calcified pulmonary nodules was noted in those who failed to react to tuberculin.

Among Indians living on the Fort Apache Agency, in the subtropical northern part of Arizona, few reacted to coccidioidin, and calcified pulmonary nodules were not observed in those who did not react to tuberculin.

Among Indians and whites living in other parts of the United States and Alaska none reacted to the injection of 0.1. cc. of a 1:1,000 or a 1:100 dilution of coccidioidin.

Sensitivity to coccidioidin is unrelated to sensitivity to tuberculin, or to proteins from other acid-fast bacteria, or to protein from *Br. abortus*.

Failure to react to tuberculin in those showing calcified nodules cannot be attributed to anergy, since in 56 of 62 such persons the tuberculin reaction became positive one year after the intracutaneous injection of BCG vaccine.

These observations suggest that the occurrence of calcified pulmonary nodules may be due to pulmonary infection with *C. immitis*.

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EFFECTS OF PROGESTERONE ON THE SEX
ORGANS AND ON THE PRODUCTION OF
PLACENTOMA IN THE FEMALE
GUINEA PIG

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AND

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In former investigations¹ it was shown that if an incision is made in the uterus of a guinea pig five to seven days after ovulation, a placentoma develops in the mucosa of this organ near the place where the cut was made. A similar effect is obtained if, instead of cutting the uterus, a foreign body, such as a glass rod, a piece of thread or paraffin, is introduced into the uterus and fixed there by ligatures. If the incision is made at an earlier period following estrus, the reaction diminishes in strength, and in the first two days after ovulation the formation of a placentoma may be lacking altogether or it may be very slight. Again, if the operation takes place later than ten days after ovulation, a placentoma does not form. Similar in principle is the reaction of the uterus of the rabbit to incision,² although the structure which develops in this species is very different from that which forms in the guinea pig; the differences are due to the differences in structure and in the potentialities of the recipient tissues of these two species.

If, instead of incising the uterus of the guinea pig, a piece of this organ is autotransplanted subcutaneously,³ a placentoma develops also in the transplanted piece, although it is somewhat smaller than that obtained in the uterus left in situ, and in the transplant the same time

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1. Loeb, L.: *Proc. Soc. Exper. Biol. & Med.* **4**:93, 1907; *Centralbl. f. allg. Path. u. path. Anat.* **18**:14, 1907; *J. A. M. A.* **50**:1897, 1908; *Centralbl. f. Physiol.* **22**:16, 1908; **24**:6, 1910; *Arch. f. Entwcklgsmechn. d. Organ.* **32**:67, 1911.

2. Loeb, L.: *Proc. Soc. Exper. Biol. & Med.* **5**:102, 1908.

3. Loeb, L.: *Arch. f. Entwcklgsmechn. d. Organ.* **31**:456, 1911.

curve is noted as in the uterus left in situ. The reaction occurs only for a certain length of time after ovulation, and it does not occur in a guinea pig in which ovulation has not previously taken place.

If incision of the uterus or transplantation of a piece of the uterus is done in an animal in which the ovaries have been extirpated or the corpora lutea have been destroyed soon after ovulation, a placentoma does not form or, if it does, it is very rudimentary; however, if the mechanical stimulus is applied about five or six days after estrus and the ovaries are extirpated shortly before the stimulation of the uterine mucosa, a placentoma may form but is diminished in size. These experiments indicate that the piece of the uterus must already be sensitized at the time when the mechanical stimulus is applied, and that, in addition, the sensitizing substance must continue to act on the uterus after the mechanical stimulation has taken place. That such sensitization takes place is indicated also by the observation that in the rabbit the epithelial changes in the uterus occur even without mechanical stimulation and that they are due to the action of the luteal hormone as such.⁴ Likewise, the treelike branching of the papillae in the uterus of the rabbit and the formation of predecidual connective tissue in the uterine mucosa of the guinea pig were shown by Ancel and Bouin⁵ and Corner⁶ to be due to the action of the corpus luteum hormone without the cooperation of mechanical stimulation. These changes occur at the height of the activity of the corpus luteum. As to the mechanical stimulus, there is reason for concluding that its effect has a relatively short duration, that it acts essentially on the mucosa of the uterus in an anteroposterior direction and that it is not the wound itself which is effective.

The lack of response of the uterus to mechanical stimuli at an early period following ovulation was explained by us as due to the fact that at this time only a small amount of luteal hormone had been able to attach itself to the mucosa and sensitize it; the lack of a reaction nine days after ovulation was attributed to either a marked decrease in the secretion of luteal hormone or a refractory state of the uterine mucosa following the period of activity.

The development of a placentoma and the activity of the connective tissue of the uterine mucosa of the guinea pig pass through four stages: (1) the formation of myxoid tissue accompanied by mitotic proliferation, (2) the formation of predecidual tissue, (3) the transformation of these actively proliferating predecidual cells into differentiated hypertrophic decidual cells and (4) the production of a definite tumor-like placentoma. As stated, in the rabbit it could be shown that not all these phases which

4. Loeb, L.: *J. A. M. A.* **53**:1471, 1909.

5. Ancel, P., and Bouin, P.: *Compt. rend. Soc. de biol.* **66**:689, 1909.

6. Corner, C. W.: *Am. J. Physiol.* **86**:74, 1928.

precede the formation of the placentoma require mechanical stimulation but that the changes in the surface epithelium of the mucosa take place under the influence of luteal hormone alone,⁴ and Bouin and Ancel⁷ found subsequently that this hormone may induce a slight production of predecidual connective tissue also in the guinea pig.

In these additional investigations we are principally concerned with a further analysis of the factors that govern the strict time limitation on the effectiveness of the luteal hormone and on the optimal result of mechanical stimulation, the optimal result extending from the fifth to the seventh day following ovulation, a time corresponding to the insertion of the fertilized ovum in the uterine mucosa. According to Weichert,⁸ these periods depend on the fact that luteal hormone is effective only if estrogen has acted on the uterine mucosa at a preceding period and that as late as ten days after ovulation the effect of estrogen discharged into the circulation has become too weak to permit successful mechanical stimulation of the uterine mucosa. His conclusions are based on experiments in which a corpus luteum extract was injected into ovariectomized rats for a period, after which mechanical stimulation of the uterus was provided, and additional injections of the corpus luteum extract were given. Under these conditions a decidual reaction of the uterine mucosa was not obtained. But if the ovariectomized rats were first brought into artificial estrus by injections of estrogen and then treated with luteal extract following stimulation, placentomas did develop. In guinea pigs ovariectomized on the fourth day after estrus, in which at the same time the uterine mucosa was stimulated, placentomas had not developed four days later; but if the corpus luteum extract was administered at the time of operation and continued to be administered for four days, a positive placentomatous reaction was obtained. If guinea pigs similarly ovariectomized were given daily injections of the corpus luteum extract from the sixth to the fourteenth day after ovulation and the mucosa was mechanically stimulated on the tenth day, placentomas did not develop. This negative result was attributed to the long interval which had elapsed since the previous estrus, in consequence of which the effect of the follicular hormone had been lost. An interpretation similar to that of Weichert was given by Brouha,⁹ Brouha and Simonnet,¹⁰ Hisaw and Leonard,¹¹ W. M. Allen¹² and Shelesnyak.¹³ Selye,¹⁴ on the other hand, in more recent experiments with 8 spayed rats found that it is

7. Bouin, P., and Ancel, P.: *Compt. rend. Soc. de biol.* **66**:505, 1909.

8. Weichert, C. K.: *Proc. Soc. Exper. Biol. & Med.* **25**:490, 1928.

9. Brouha, L.: *Proc. Soc. Exper. Biol. & Med.* **25**:488, 1928.

10. Brouha, L., and Simonnet, H.: *Compt. rend. Soc. de biol.* **99**:1926, 1928.

11. Hisaw, F. L., and Leonard, S. L.: *Am. J. Physiol.* **92**:574, 1930.

12. Allen, W. M.: *Am. J. Physiol.* **100**:650, 1932.

13. Shelesnyak, M. C.: *Anat. Rec.* **56**:211, 1933.

14. Selye, H.: *Proc. Soc. Exper. Biol. & Med.* **43**:343, 1940.

possible to obtain predecidual changes by the use of progesterone alone, and if, in addition, mechanical stimuli are applied, placentomas develop without previous injection of an estrogen.

In our experiments, which were begun more than six years ago, we studied the effects of progesterone, with and without the addition of an estrogen, and also the effects of combinations of progesterone and an extract of the anterior lobe of the hypophysis, as well as combinations of these agents and mechanical stimulation of the uterus. The experiments now reported differed from our earlier ones in that special attention was given to the predecidual changes which occurred in the uterine mucosa, in addition to the formation of placentomas.

MATERIAL AND DOSAGE OF PROGESTERONE

One hundred thirty female guinea pigs were used in these investigations. They were immature at the beginning of the experiment, most of them weighing

TABLE 1.—*Doses of Progesterone Used in the Experiments*

Dose of Progesterone in Oil, Cc.	International Rabbit Units of Progesterone	Corner and Allen Rabbit Units of Progesterone
0.04	0.20	0.15
0.07	0.35	0.26
0.10	0.50	0.38
0.13	0.65	0.49
0.17	0.85	0.64
0.20	1.00	0.75

between 180 and 200 Gm.; it is to be noted, however, that in several of the series in which we compared the reaction of ovariectomized and nonovariectomized guinea pigs, the ovariectomized animals weighed somewhat less at the time of operation.

Six different amounts of the preparation of progesterone used¹⁵ were administered in the five series of experiments. Table 1 shows these doses and the units which they represent.

METHOD OF PRESENTATION

In describing the reactions in the uterine mucosa, we shall distinguish several stages: (1) the formation of myxoid connective tissue in which mitoses occur; this leads to (2) the production of predecidual tissue, in which the fibroblasts are increased in size and the collagenous fibrils are very much diminished or lacking; (3) the development of areas composed of decidual cells, which are larger and which often assume a polygonal shape, and (4) in the end the mitotic proliferation and the hypertrophy of the cells leading to the formation of a placenta in which, in the older portions of the tissue, often pearl-like structures occur, while in the peripheral parts, around vessels, some strands of cells may still remain in the less differentiated state of predecidual tissue.

15. Dr. Erwin Schwenk, of the Schering Corporation, supplied the progesterone in oil (proluton) employed in these experiments.

SERIES I

INJECTION OF VARIOUS DOSES OF PROGESTERONE INTO GUINEA PIGS
WITH INTACT UTERUS

Nonovariectomized Guinea Pigs.—Eleven guinea pigs were given daily injections of progesterone in oil for a period of six days, the doses being as follows: 0.07 cc., 0.10 cc., 0.13 cc., 0.17 cc. and 0.20 cc. Examination took place on the day following that of the last injection. With 0.07 cc. the effects on the uterine mucosa were negative or very slight. There were noted some edema and myxoid (and occasionally also predecidual) connective tissue with mitoses and with enlargement of the connective tissue cells. Likewise, in animals given somewhat larger doses either there was a formation of myxoid tissue only or there was a predecidual reaction. With still larger doses (0.17 cc. and 0.20 cc.), a more distinct predecidual connective tissue developed in some places, while in others the connective tissue had merely a myxoid character. Only in a single animal, which had received 0.10 cc. of progesterone in oil, was there beginning formation of decidual tissue in addition to the predecidual reaction. With a longer course of injections, the reaction did not become more marked as one would have expected; on the contrary, it tended to diminish. After twelve, sixteen, twenty-three or forty-eight injections,¹⁶ one injection being given daily, as a rule merely myxoid and edematous connective tissue developed; likewise, mitoses were usually lacking at later periods. Whenever mitoses were present, they were found in the gland fundi as well as in the connective tissue, although in some instances they occurred in the gland fundi alone.

As to the effect of progesterone on the vagina, mitotic proliferation of the layer of cuboidal cells was observed in only 2 of the guinea pigs, the first of which had been given 0.10 cc. of progesterone in oil daily for six days, and the second, 0.17 cc. for twelve days. In these animals there was also a predecidual reaction in the uterus. However, the effect in the vagina may have been due to the presence of large follicles in the ovaries of these guinea pigs. The upper layer of cylindric epithelium was usually not higher than that in nontreated normal controls with intact ovaries, but in some animals, treated for longer periods, the cells seemed to stain a deeper blue with hematoxylin, indicating perhaps an intensified tendency toward production of mucin.

In the mammary gland there was noticeable, as a rule, some mitotic activity, which affected either epithelium or connective tissue. In animals treated for longer periods or with larger doses, these effects appeared to be greater than after injections for only six days.

In the ovaries there were usually large, well preserved follicles, and in some cases even mature follicles, whereas corpora lutea were lacking.

Ovariectomized Guinea Pigs.—In ovariectomized guinea pigs given 0.04 cc. or 0.07 cc. of progesterone in oil daily for six or eight days, the reaction in the uterine mucosa was very mild; the connective tissue was myxoid or almost predecidual and showed an increase in mitoses. The latter type of change affected also the muscle tissue. Much edema was observed in the deeper tissues, where the larger vessels were situated, and especially between the muscle layers. In a few animals a predecidual and even a decidual reaction was obtained. On the

16. Ten of these guinea pigs served also in experiments of Dr. Martin and Dr. Ruth Silberberg on the effects of progesterone on bones.

whole, the reactions in the uterus were similar to those noted in the non-ovariectomized animals under comparable conditions.

Guinea pigs treated from nine to sixteen days with doses of progesterone in oil varying between 0.04 cc. and 0.17 cc. showed either moderate predecidual reactions or only the development of myxoid connective tissue. In guinea pigs treated for twenty, twenty-three or twenty-five days with 0.13 cc. or 0.17 cc. of progesterone in oil, there was either myxoid connective tissue or merely fibrillar-cellular connective tissue, and in some instances, a slight increase in mitoses. On the whole, therefore, the results in ovariectomized guinea pigs were similar to those observed in nonovariectomized animals, and in both types the reactions did not increase in strength with a longer course of injections; on the contrary, there was some indication that they became less intense and that a refractory condition developed in the uterus.

In the vagina the cuboidal epithelium was, in general, in a resting condition; in only a single animal, treated for twenty-three days, were a few mitoses found; however, in several guinea pigs a few mitoses were present in the layer of vacuolated cylindric cells of the vagina or cervix, and these cells were, in general, higher than those in nontreated ovariectomized controls.

While in the large majority of the nonovariectomized guinea pigs the mammary gland showed proliferation, it was, as a rule, in a resting condition in most of the ovariectomized animals.

SERIES II

INJECTION OF A COMBINATION OF PROGESTERONE IN OIL AND ANTERIOR HYPOPHYSIAL EXTRACT INTO GUINEA PIGS WITH INTACT UTERUS

Nonovariectomized Guinea Pigs.—Seven guinea pigs were given daily subcutaneous injections of progesterone in oil for six days, the doses being 0.07 cc., 0.13 cc., 0.17 cc. or 0.20 cc.; they also were given for six days intraperitoneal injections of 1 cc. doses of an anterior hypophysial extract prepared in our laboratory. Examination took place on the day following that of the last injection. In 3 of these animals the mucosa of the uterus showed myxoid connective tissue, in which only a few mitoses were seen; there was edema in the deeper areas of the stroma between the gland fundi and also underneath the epithelium. In 4 guinea pigs there was a predecidual reaction, but in only a single animal, receiving 0.17 cc. of progesterone in oil daily, was this reaction considerable. In 2 of the 7 animals the reactions were similar to those seen in the controls given progesterone in oil alone, while in 5 they were somewhat less marked.

Two guinea pigs were given daily for twelve days 0.13 cc. or 0.17 cc. of progesterone in oil and 1 cc. of anterior hypophysial extract. There was no predecidual reaction in the uterine mucosa, but myxoid connective tissue was found without or with only a few mitoses. One of these animals had a uterine reaction similar to that seen in the control, while in the other the reaction was less; in addition, the uterine mucosa in one of these animals showed some signs of disintegration of connective tissue cells which formerly had proliferated, and it seemed that the disintegrated particles were taken up by phagocytes.

It may be concluded that the injection of the hypophysial extract did not increase the intensity of the reaction of the uterine mucosa, but that, if any change occurred, it consisted rather in a tendency toward diminution of this reaction.

Proliferation of the vagina was not observed. Proliferation of the mammary gland was present in 3 guinea pigs; in 3 others, receiving either 0.17 or 0.20 cc.,

the mammary gland was in a resting condition. The ovaries showed a typical atretic effect characteristic of the influence of injections of hypophysial extract. It is possible that the relatively inactive state of the ovaries in these animals was responsible for the diminution in the reaction of the uterine mucosa.

Ovariectomized Guinea Pigs.—Six ovariectomized guinea pigs received 0.04, 0.07, 0.13 or 0.20 cc. of progesterone in oil and 1 cc. of anterior hypophysial extract intraperitoneally daily for six days. In 3 of these animals a predecidual reaction was obtained, while in the 3 others only myxoid connective tissue developed. In general, mitoses were found in cells which were enlarged and in places where the development of fibrillar connective tissue was diminished or lacking. In an additional ovariectomized animal, 0.13 cc. of progesterone in oil and 1 cc. of anterior pituitary extract were injected daily for fourteen days, and in another, for twenty days. No mitoses were seen in the animal treated for twenty days, and here the connective tissue had assumed largely an inert fibrillar character. There was no direct relation between the strength of the reaction and the dose of progesterone administered. In 2 of the guinea pigs in this series, edema was present, and there were indications of the same stratification of the connective tissue which had been seen in other experiments; in the deepest zone there was marked edema, while above this there was a layer of predecidual tissue, and underneath the epithelium there was again a myxoid or slightly edematous area. In 3 of these animals the reaction was slightly stronger than in controls given the same dose of progesterone. One of them had a reaction of about the same strength, and in 2 it was somewhat stronger than that of the controls, which did not receive hypophysial extract. In ovariectomized guinea pigs hypophysial extract was therefore without any noticeable effect on the uterus; this result may perhaps be interpreted as a confirmation of the conclusion that if the extract exerts any effect at all, it does so by way of the ovaries.

As to the vagina, a slight mitotic proliferation of the flat cuboidal layer was noted in the animals treated for the longest time (fourteen and twenty days). The upper cylindric layer was higher than in nontreated ovariectomized controls, and mitoses were found in this layer in a guinea pig given 0.04 cc. of progesterone in oil for six days and in another given this dose for twenty days. The mammary gland was in a resting state similar to that found in controls in all except a single instance, in which a few epithelial mitoses were seen.

As usual, the injection of the extract of the anterior lobe of the hypophysis tended to lower the weights of the guinea pigs as compared with those of nontreated controls, owing to the stimulation it produces on the thyroid gland.

SERIES III

INJECTION OF VARIOUS DOSES OF PROGESTERONE INTO GUINEA PIGS IN WHICH AN INCISION HAD BEEN MADE IN THE UTERUS OR A GLASS ROD HAD BEEN INSERTED INTO ONE OF THE UTERINE HORNS

Nonovariectomized Guinea Pigs.—The procedure in these experiments, in which 6 guinea pigs were used, was as follows: In a first period of six, fifteen or twenty-three days 0.13 cc. or 0.20 cc. of progesterone in oil was injected daily. On the day following that of the last injection a part of one uterine horn was slit open, while a glass rod was inserted in the other horn, where it was kept in position by means of ligatures applied at the upper and lower ends. In a third period each animal received a series of daily injections of the same amount of progesterone in oil as given during the first period.

In the 2 guinea pigs in which the first period of treatment lasted six days and the doses injected were 0.13 cc. and 0.20 cc. of progesterone in oil, placentomas developed in some places in the uterine mucosa, and in others there were transitions between myxoid, predecidual and decidual reactions. As usual, mitoses were seen, especially in the smaller, less differentiated decidual cells. In each of these animals the reaction was somewhat less marked in the place where the glass rod had been inserted than in the place where the uterus had been slit. At a distance from the incision, in the noncut portion of the uterine horn, the reaction was slight. In the guinea pig treated for a first period of fifteen days with 0.20 cc. of progesterone in oil, a placenta developed neither in the incised horn nor in the horn containing the glass rod; instead, slightly stimulated fibrillar connective tissue had formed, and in only one place, under the influence of the glass rod, predecidual connective tissue was found. The reaction was likewise very much weakened in the 2 guinea pigs given 0.13 or 0.20 cc. of progesterone in oil during a first period of over twenty-three days. As a rule, the connective tissue here was fibrillar and in places slightly edematous, and the cells were arranged with their long axes parallel to the surface epithelium. Mitoses were lacking or infrequent. Placentomas did not develop, but in contact with the glass rod a small amount of predecidual tissue had formed.

From these experiments we may conclude that the continued stimulation exerted by an incision or a foreign body intensifies very much the proliferative reaction of the uterine mucosa to the progesterone injected but that a prolongation of the period of injections tends to weaken this reaction under these conditions.

The cuboidal epithelium in the vagina was in a resting state in all of these guinea pigs. The mammary gland showed proliferation only in the guinea pigs treated for twenty-three days. It is probable that the long-continued administration of progesterone had a stimulating effect on the mammary gland. In the ovaries, as a rule, well preserved follicles, but no corpora lutea, were visible.

Ovariectomized Guinea Pigs.—In 8 guinea pigs 0.04 cc., 0.13 cc. or 0.20 cc. of progesterone in oil was injected daily for six days; the following day the horn of the uterus was slit and in 4 of these animals a glass rod was inserted into the second horn. This was followed by a period of injections lasting five or six days, with the same amount of progesterone in oil being given as in the first period. In 3 of these guinea pigs, given 0.04 cc. of progesterone in oil, there was predecidual connective tissue in the mucosa of the uterus, and in some papillae elsewhere beginning placentomatous areas and many mitoses were found in the connective tissue. In a fourth guinea pig, treated in the same way, the connective tissue was fibrillar or myxoid; however, predecidual connective tissue was lacking. With a dose of 0.13 cc. of progesterone in oil, either a predecidual reaction or the formation of placenta took place; away from the place of mechanical stimulation the mucosa showed merely a myxoid reaction. A daily dose of 0.20 cc. of progesterone in oil gave rise, near or at some distance from the place of incision, to the formation of an extensive placenta or of several smaller placentomas, containing typical pearls; and even at a distance from the cut some decidual tissue formed in addition to myxoid and predecidual connective tissue. In certain instances there was apparently a parallelism between the gain in weight which had taken place during the time of the experiment and the intensity of the reaction, the reaction being weaker in the animal in which the gain had been less; such a connection between weight and intensity of reaction was not, however, noticeable in all the animals.

In four experiments, 0.33 cc. of 0.9 per cent sodium chloride solution was injected during a first period; on the day after that of the last injection, one horn of the uterus was incised and a glass rod inserted in the other horn, and then 0.04 or 0.07 cc. of progesterone in oil was injected daily for six more days. With 0.04 cc. as well as with 0.07 cc. of progesterone in oil, a predecidual reaction took place, and around the glass rod a beginning decidual reaction developed at some points. Many mitoses were seen in the predecidual tissue, except in places where marked edema had injured the cells. In 1 guinea pig the reaction proceeded only to the formation of predecidual tissue. In the vagina the vacuolated cylindric epithelium of the surface was higher than in nontreated ovariectomized guinea pigs. In only 1 guinea pig were a few mitoses found in the epithelium of the mammary gland.

These experiments were varied in several ways. Addition of a series of injections of sodium chloride solution, either preceding or following the first series of injections of progesterone in oil, tended to weaken the reaction, perhaps by affecting unfavorably the state of nourishment of the guinea pig. An increase in the dose of progesterone usually intensified the reaction. If the last series of injections of progesterone in oil was omitted, the reaction was very much weakened. In 6 guinea pigs the dose of progesterone in oil during the first period was increased to 0.13 and 0.17 cc. and at the same time the period of injections was prolonged to ten, fourteen, fifteen or twenty-three days. With a dose of 0.13 cc. and a fourteen day first period, placentoma formation still took place; after injections for sixteen days, only a combination of predecidual and myxoid connective tissue developed, and after injections for twenty-three days, myxoid connective tissue alone. With a dose of 0.20 cc. of progesterone in oil given daily for fifteen days, myxoid connective tissue predominated, but some tissue intermediate between predecidual and decidual was seen; if the administration of this amount of progesterone in oil lasted twenty-three days, only the production of fibrillar and myxoid connective tissue took place. In these experiments, the prolongation of the first period of injections had to be greater to diminish the effect than in the former experiments, in which a mechanical stimulation of the uterus had been omitted.

The following conclusions may be drawn from these experiments: Mechanical stimulation of the uterus in combination with progesterone action intensifies considerably the effect of the progesterone. In both nonovariectomized and ovariectomized guinea pigs, injections of 0.13 cc. and 0.20 cc. of progesterone in oil for two periods of six and five days, respectively, one preceding and the other following the mechanical stimulation of the uterus, lead to the formation of placentomas. If the first period of injections is extended to fifteen, sixteen or twenty-three days, placentomas do not form, the reaction being the weaker the longer the first period of injections. A prolongation of the period of injections increases the height of the vacuolated cylindric epithelium of the vagina in ovariectomized guinea pigs as compared with that in nontreated controls, and it also may induce some proliferation of the mammary gland.

Injections of smaller doses of progesterone in oil (0.04 cc. and 0.07 cc.), given under similar conditions for a first period of six days and after operation for a second period of five days, did not lead to the production of placentomas but to the formation of predecidual or merely myxoid connective tissue. A prolongation of the first period of injections to sixteen days did not noticeably weaken the reaction. It is possible that the mechanical stimulation of the uterus counteracted to some extent the effect of the prolongation of the course of injections, which

tends to induce a refractory state. However, it cannot be excluded that a still greater increase in the period of injections would have diminished more definitely the effectiveness of progesterone also in this series. In experiments in which the last period of injections was omitted and the progesterone in oil was injected only just before the mechanical stimulation of the uterus, only a very slight reaction or none followed. On the contrary, if the first series of injections was omitted and progesterone in oil was injected only during the period following the mechanical stimulation of the uterus, predecidual tissue or tissue intermediate between predecidual and decidual tissue did develop. It is the last series of injections of progesterone in oil which seems to be the more effective one.

SERIES IV

INJECTIONS OF BOTH PROGESTERONE AND ESTROGEN INTO GUINEA PIGS WITH INTACT UTERUS

Nonovariectomized Guinea Pigs.—Two guinea pigs each received a single subcutaneous injection of 100 rat units of estrogen; this was followed by daily subcutaneous injections of 0.07 cc. and 0.13 cc. of progesterone in oil for six days. The reactions in the uterus were, on the whole, similar to those observed in the controls receiving the same amount of progesterone in oil without estrogen. However, the thickness of the mucosa was perhaps greater in some places in this series, owing probably to increased edema. A variable number of mitoses were seen in the connective tissue and, as in many other instances, mitoses were found also in the muscle layers of the uterus. In general, the reaction led only to the formation of myxoid connective tissue, but there was a slight amount of predecidual tissue in some places. In the vagina the vacuolated cylindric cells were high, but no proliferation had occurred in the cuboidal cells. In the mammary gland in one guinea pig a few mitoses were seen, a condition similar to that in the control; in the other guinea pig there was much secretion. In the ovaries, preserved, large follicles were found.

Ovariectomized Guinea Pigs.—A repetition of the experiment just described, but in previously ovariectomized instead of in nonovariectomized guinea pigs, led essentially to the same results as far as the uterine mucosa was concerned. There was a slight myxoid reaction with mitoses in the connective tissue in the animal treated with 0.07 cc. of progesterone in oil, and there was a slight predecidual reaction in the guinea pig given 0.13 cc. of progesterone in oil. The proportion of solid predecidual tissue as well as of edema was greater in these animals than in the control. With an increase in the amount of progesterone in oil, the reaction became stronger. In one case there was an increase in the height of the vacuolated cylindric epithelium in the vagina. The mammary gland was resting, as it was in the ovariectomized controls. We may conclude, then, that in both the nonovariectomized and the ovariectomized guinea pigs the uterine reaction was slight but that it increased somewhat in strength with an increase in the dose given. Administration of a single large dose of estrogen, therefore, did not alter much the reaction to progesterone in the uterine mucosa.

In a second experiment with ovariectomized guinea pigs, instead of giving a single dose, 4 or 8 rat units of estrogen was injected daily on four successive days. This was followed by daily injection of either 0.04 cc. or 0.07 cc. of progesterone in oil for a period of six days. The uterine mucosa responded to these injections with the formation of myxoid or predecidual connective tissue; as in former experi-

ments, the reaction was stronger with 0.07 cc. than with 0.04 cc. of progesterone in oil. The effect with estrogen was similar to that noted in control animals not receiving estrogen, but on the whole it was somewhat more marked, the number of mitoses being greater and the number and the size of predecidual cells being slightly increased; edema, however, was perhaps less pronounced than in the controls not receiving estrogen. With 8 rat units of estrogen the effects were somewhat greater than with 4 rat units. There were mitoses also in the muscle cells. While on the whole there is, then, in these experiments, a difference in favor of the guinea pigs receiving estrogen in addition to progesterone, the difference is slight, and in no case was the myxoid and the predecidual reaction changed to a placentomatous reaction.

In the vagina, the addition of estrogen to progesterone treatment had a definite effect. In the upper layer there were either high vacuolated cylindric cells or vacuolated and hyalinized cells alternated. Furthermore, in 2 of these animals mitoses appeared in the upper cells. With 8 rat units there was a proliferation of cells in the cuboidal layer, attributable mainly or entirely to the estrogen given. With 8 rat units there was also some proliferation of the mammary gland, a reaction which was lacking with 4 rat units.

In a third experiment, 3 guinea pigs were given daily injections of 0.04 cc. of progesterone in oil for sixteen days; this was followed by daily injections of 30 rat units of estrogen for four days. The examination took place two, four or six days subsequent to the last injection of estrogen. In the uterine mucosa of these guinea pigs a predecidual reaction was lacking; there was merely a formation of myxoid connective tissue, with or without mitoses. How much of the depression in the reaction of the uterine mucosa was due to the prolongation of the administration of progesterone and how much to the time which had elapsed since its administration before examination was made remains to be investigated. In the vagina there was squamous epithelium, with or without papillae formation, in the vacuolated cylindric surface epithelium. There was no proliferation in the mammary gland.

In general, it may be stated that estrogen may or may not intensify the action of progesterone on the uterine mucosa, but such an augmenting effect, if present, is slight and is not able to bring about a placentomatous reaction in these animals. As in our previous experiments, larger amounts of progesterone are more effective than smaller ones. In accordance with expectations, estrogen produces proliferation of the cuboidal epithelium in the vagina, leading to the formation of several layers of squamous epithelium, with or without the addition of an upper layer of hyaline or vacuolated epithelial cells.

SERIES V

INJECTIONS OF BOTH PROGESTERONE AND ESTROGEN IN OVARECTOMIZED GUINEA PIGS, IN WHICH AN INCISION WAS MADE IN ONE HORN OF THE UTERUS AND A GLASS ROD INSERTED IN THE OTHER

In this series, the combined effects of progesterone and estrogen on the uterus, the vagina and the mammary gland were compared with those occurring in animals which received progesterone alone and in those to which injections of 0.9 per cent sodium chloride solution were given instead of injections of estrogen. Twenty-four guinea pigs were used in the eight experiments comprising this series.

Experiment 1.—In 1 guinea pig the smallest dose of progesterone in oil (0.04 cc.) was injected daily for eight days, then 8 rat units of estrogen was injected daily

for four days. In 4 guinea pigs the sequence was reversed, estrogen being injected first, followed by injections of 0.04 cc. of progesterone in oil. In all of these animals the uterus was then cut and 0.04 cc. of progesterone in oil was injected for four additional days. There was a moderate predecidual reaction in the uterus of the first guinea pig and some placentomatous nodules developed in 3 guinea pigs in which estrogen was first injected, but in the fourth animal only predecidual tissue was found. The reaction was intensified in 3 of the 4 animals receiving estrogen first, but not in the first case in which the administration of estrogen followed the initial series of injections of progesterone in oil. In the vagina there was hyalinization of some of the upper cells or marked vacuolation and some mitoses in other cells of the upper layer in 2 of the animals treated first with estrogen, a condition not found in the control animals. In the other 2 guinea pigs which received estrogen previous to the administration of progesterone, high cylindrical epithelium without mitoses was found. In the guinea pig treated first with progesterone, the vagina was in a resting state. The mammary gland behaved in the same way as in controls; there was either a slight stimulation or a state of rest.

Two additional guinea pigs were treated in the same way, except that 0.13 cc. or 0.20 cc. of progesterone in oil was injected daily following a first series of injections of estrogen. In the incised uterine horns of these guinea pigs large placentomas developed; around the glass rod also a large placenta was found in one animal and only beginning decidual tissue in the other. The cuboidal epithelium of the vagina was in a resting state in both guinea pigs.

Experiment 2.—Four guinea pigs were used. Thirty rat units of estrogen was injected daily for four days; the usual operation on the uterus was then performed, and this was followed by daily injections of 0.04 cc. or 0.07 cc. of progesterone in oil for six days. In the uterine mucosa, predecidual tissue developed in the incised horn, and, in addition to the predecidual tissue, beginning formation of decidual tissue occurred under the influence of the glass rod. The predecidual reaction extended to places at a distance from the area of incision in the horn without the glass rod. With 0.07 cc. of progesterone in oil the reaction was slightly stronger than with the smaller dose. On the whole, the effects were similar to those seen in the controls, in which estrogen had not been injected, but the addition of the latter substance caused perhaps a slight intensification. With 0.07 cc. of progesterone in oil, two layers of cuboidal epithelium developed in the vagina, while in the animals receiving the smaller dose there were hyaline as well as mucoid cells in the upper layer of the epithelium, accompanied by mitoses and amitoses. In these guinea pigs the mammary gland behaved similarly to that in the controls; there was either a very slight or no stimulation.

Experiment 3.—A daily injection of 0.04 cc. or 0.13 cc. of progesterone in oil was given for sixteen days. This was followed by daily injections of 8 rat units of estrogen for four days; then the uterus was incised, a glass rod introduced, and 0.04 cc. of progesterone in oil was injected daily for four or five additional days. As usual, examination took place on the day following that of the last injection. With 0.04 cc., only myxoid connective tissue, in which there were a few mitoses, was noted in the uterus; with 0.13 cc. of progesterone in oil, a predecidual reaction occurred; in both instances the reactions were somewhat less marked than in controls not receiving estrogen. Either the smaller effects in these cases were caused by the reversal of the order in which estrogen and progesterone were given, or the differences were merely accidental. As also in the control, the cuboidal epi-

thelium of the vagina was in a resting condition with the small dose of progesterone, and there was perhaps a slight mucoid reaction of the upper layer with the larger doses. In the mammary gland no stimulation was observed. The prolonged course of injections of progesterone in oil in the first period or the relatively small amount of estrogen given, or both of these factors, prevented the stimulating effect on the mammary gland tissue which estrogen otherwise tends to exert.

Experiment 4.—A daily injection of 0.07 cc. of progesterone in oil was given for sixteen days and then a daily injection of 30 rat units of estrogen was given for four days. Incision and insertion of a glass rod in the uterine horn followed, and 0.07 cc. of progesterone in oil was injected daily for the following five days. In the uterus a predecidual or early decidual reaction was noted, which extended also into places at some distance from the incision. The effects were somewhat similar to or slightly stronger than those seen in the controls. In the vagina there was either a stimulation of the upper cell layer, as indicated by a somewhat increased height of the vacuolated cylindric cells, or a slight proliferation of the cuboidal layer; the latter was due to estrogen. In the mammary gland a resting condition was found, much like that seen in the controls, or there was a slight stimulation of epithelium and connective tissue, which was lacking in the controls. Again, this effect must in all probability be attributed to the administration of estrogen.

Experiment 5.—The procedure and in particular the quantities of progesterone and estrogen used in this experiment were the same as in experiment 4 except that the injections of estrogen were given subsequent to and not preceding the incision in the uterus and the introduction of the glass rod. The reactions were about the same as in the control, or they were a little stronger. There was formation of well developed predecidual tissue and beginning decidual reaction in places. Predecidual tissue in the mucosa was observed also at some distance from the place of incision. Injections of estrogen following the operation seemed to cause a stronger mucification of the upper epithelial layer of the vagina and a greater stimulation of the lower (cuboidal) epithelium. As might be expected, the later date of the injections of estrogen caused a stronger response in the vagina. In the mammary gland no stimulation was noticeable; if such an effect had occurred at an earlier period, it evidently had passed off at the time of examination.

Experiment 6.—This experiment was the same as experiment 5 except that 0.04 cc. instead of 0.07 cc. of progesterone in oil was injected. In the incised region of the uterus as well as at some distance from the place of incision there was a predecidual reaction, and in contact with the glass rod an early decidual reaction had developed. In most places the reaction was somewhat stronger than that in the controls, which had received progesterone without estrogen, but in other places the reaction was about the same. In the vagina, estrogen induced a proliferation of the cuboidal cell layer as it did in experiment 4. The mammary gland was in a resting state.

Experiment 7.—The procedure was the same as in experiment 4 except that 0.04 cc. instead of 0.07 cc. of progesterone in oil was injected. In the uterine mucosa at the site of the incision, as well as in contact with the glass rod, and even at some distance from the place of incision, a definite predecidual reaction had developed, and in some places decidual changes were seen. The reactions were somewhat more marked than those in the controls, not given injections of estrogen, and they were likewise stronger than in those experiments in which the injections of estrogen followed the uterine operation. In the vagina, estrogen caused a mild

additional stimulation of the upper cylindric layer, which was higher than that in the controls and in which some mitoses were noted. In certain of the animals there was also a slight stimulation of the cuboidal epithelium, so that two layers of epithelial cells lined the vaginal cavity. In the mammary gland the epithelium and the connective tissue proliferated in 1 guinea pig, but a resting condition was noted in the others. Similar variations in the mammary gland had been found also in experiment 4.

Experiment 8.—In this experiment the second period of injections of progesterone in oil, the one following the operation, was omitted. The first period of injections of 0.04 cc. of progesterone in oil extended over sixteen days; 30 rat units of estrogen was administered daily for the following four days, and this was followed by the operation; autopsy was performed one, three or five days after the operation. In all 3 of these guinea pigs the reaction in the uterus was very slight; the mucosa consisted largely of fibrillar or myxoid connective tissue. However, in the first guinea pig, examined one day after operation, there was a slight amount of predecidual connective tissue near the place of incision; in the second animal, examined three days, and in the third, examined five days, after the operation, the predecidual stage had not yet been quite reached. It seems necessary, then, to administer progesterone after the operation has been carried out in order to obtain a strong reaction; without a second series of injections of this agent only very imperfect results are obtained, a conclusion which is in accordance with our previous observations. In the vagina the effects of the injections of estrogen were definite. After one day there was squamous epithelium with keratin; after three days the keratin had been cast off, and after five days there were only two to three layers of squamous epithelium, covered by hyalinized cells. The estrogen also stimulated the mammary gland, a stimulation which was strongest after one day, when mitoses were found in the epithelium and connective tissue. After three days, mitoses were found mainly in the epithelium of the ducts, and after five days the stimulation had passed; mitoses no longer were visible. There was, then, a noticeable parallelism between the effects in the vagina and the mammary gland.

In general, it may be stated that in guinea pigs the effects of injections of progesterone on the uterine mucosa are not influenced to any marked degree by addition of injections of estrogen. In a number of animals, throughout the uterine mucosa, and in others in certain parts of it, the reaction was about the same as in controls, to which estrogen had not been administered. In a few guinea pigs the reaction was weaker than in the controls; but in the majority of experiments the reaction was somewhat intensified through addition of estrogen. However, as stated, a marked change in the reaction was not brought about by the addition of estrogen. Furthermore, it seems that the series of injections of progesterone which follows the mechanical stimulation of the uterus is a very important factor in determining the strength of the reaction in the uterine mucosa. On the other hand, in the vagina and the mammary gland it is the estrogen and the time of its administration which are more potent in producing proliferation, although, as seen in ovariectomized guinea pigs, progesterone, as such, is able to exert some effect in the vagina, inasmuch as it causes an increase in the height of the upper

vacuolated layer of the epithelium, as compared with the findings in non-treated ovariectomized controls. On the whole, it may be stated that the larger the doses of progesterone and of estrogen, the more marked were the results in the uterus. However, this is not the case if the time during which the first series of injections of progesterone is given is prolonged; such a prolongation does not lead to an intensification of the effects in the uterus; on the contrary, it tends to weaken them. As regards the vagina, we observed, in addition to the stimulation of the cuboidal cell layer, which has been found in a number of cases mainly under the influence of estrogen, an increase in the height of the upper layer of epithelium in ovariectomized guinea pigs, and in some instances a mucoid condition or hyalinization and mitotic proliferation of the upper layer of cells.

COMMENT

The fact that the administration of estrogen in addition to and preceding the injections of progesterone either did not noticeably increase the intensity of the reaction of the uterine mucosa to the latter substance, or increased it to only a slight degree, does not seem to support the conclusion that progesterone is active only in cooperation with estrogen. Likewise incompatible with this conclusion is the demonstration that it is possible to produce a definite predecidual reaction in animals in which the uterus has not been mechanically stimulated, and to produce placentomas following mechanical stimulation, in ovariectomized as well as in non-ovariectomized guinea pigs, by the use of progesterone alone, without the addition of estrogen. However, our experiments have shown that a moderate intensification of the predecidual reaction may occur in cases in which estrogen is injected in addition to progesterone. The latter result can readily be understood if one considers the fact that estrogen alone exerts a slightly stimulating effect on the uterine mucosa. A summation of the effects of estrogen and progesterone on the uterine mucosa might, therefore, be expected to take place, and this would satisfactorily explain the results obtained in these experiments.

As to the loss of effectiveness of the mechanical stimulation during the sexual cycle when the incision is made or a foreign body is introduced ten days or more after estrus, this is probably due either to a diminution in the amount of progesterone discharged into the circulation at that time or to the gradual development of a refractory state in the uterine mucosa as a result of the preceding period of stimulation. This would be in harmony with the interpretation given by Loeb¹; it would also be in harmony with our recent finding that long-continued treatment with progesterone, instead of intensifying the response of the uterine mucosa, on the contrary, tends to diminish it. It may therefore be considered proved that it is possible to produce a state of partial or complete refrac-

toriness to progesterone by long-continued administration of this substance, a result similar to that previously obtained in the thyroid gland of the guinea pig after prolonged injection of iodine and after extended treatment with a thyroid-stimulating extract of the anterior lobe of the pituitary gland.¹⁷ Turner and Frank,¹⁸ and also Selye,¹⁹ have made similar observations in the case of estrogen. In view of the chemical structure of progesterone, this refractory state cannot be attributed to the production of antibodies.

As to the effect of progesterone on the vagina, it could be shown that in ovariectomized guinea pigs this substance caused an increase in the height of the epithelial layer lining the cavity, consisting normally of vacuolated cylindric cells. In a few instances, after long-continued treatment with progesterone mitotic proliferation of these cells could be observed, and occasionally mitoses were noted even in the layer of cuboidal epithelium. It seems, then, that progesterone not only stimulates the uterus but may exert a certain influence also on the epithelium of the vagina. The effects of progesterone on the cylindric layer of the vaginal epithelium could be demonstrated definitely only in ovariectomized animals, owing to the fact that in nonovariectomized guinea pigs, as a rule, cyclic changes began in the vaginal epithelium before the conclusion of the experiments if the injections were extended over a long period and that these cyclic changes introduced complications which made the results less clearcut.

In some instances it seemed that stimulation by progesterone was exerted also on the mammary gland tissue, as indicated by the appearance of mitoses in the epithelial structures and, in certain cases, also in the connective tissue stroma; however, these effects were inconstant and at best not very pronounced.

The foregoing observations concerning the action of progesterone on the various sex organs have been shown to apply at present only in the case of guinea pigs. However, it is very probable that in principle the conditions in other species are similar, although differences exist in different species, at least in two respects: First, the recipient organs in various species respond in specific ways to the action of the same kind of hormone, and second, the organ producing the hormone may show differences in different species. There are no data which indicate differences in the chemical constitution of progesterone in species as nearly related as the guinea pig, the rat and the rabbit. The response

17. Loeb, L.: *Science* **80**:252, 1934.

18. Turner, C. H., and Frank, A. H.: The Effect of the Ovarian Hormones Theelin and Corporin upon the Mammary Growth of the Rabbit, *Research Bulletin* 174, University of Missouri, College of Agriculture, Agricultural Experiment Station, 1932.

19. Selye, H.: *Am. J. Physiol.* **130**:358, 1940.

of the uterine mucosa in the rat and that in the guinea pig to this hormone are very similar; there are, however, differences of character in the sexual cycles of these two species, which are largely due to different degrees of resistance to injurious conditions on the part of the corpus luteum; the latter is very much more resistant in the guinea pig. The reverse condition exists in the case of the follicles, which give origin to the corpus luteum; these possess a greater resistance in the rat.²⁰ These two factors are largely due to the difference in the duration and in the other characteristics of the sexual cycle. The difference in the resistance of the corpora lutea explains also the difference in the effects of hysterectomy on the sexual cycle in the rat and the guinea pig. However, these variations in the function of the corpus luteum in the rat and the guinea pig seem to be mainly of a quantitative nature.

SUMMARY

Progesterone injected in sufficient doses into immature ovariectomized and nonovariectomized guinea pigs called forth a predecidual or a decidual reaction in the uterine mucosa; the reaction was intensified with an increase in the amount of the substance administered within the range of doses used by us.

Injections of anterior pituitary extract added to those of progesterone did not intensify the reaction but, on the contrary, seemed to diminish it in animals with intact ovaries.

Mechanical stimulation of the uterine mucosa by incision into a uterine horn or by introduction of a foreign body into the uterine cavity intensified the reaction of the uterine mucosa and made possible the production of placentoma. Injections of progesterone were more potent in producing this effect if made after rather than before mechanical stimulation.

Estrogen administered before progesterone as a rule intensified somewhat the effects of the latter. This intensifying effect was, however, slight in these experiments. On the whole, it was perhaps due to a summation of the stimulation exerted by each one of these substances separately; in some instances it was lacking. Estrogen was usually ineffective if injected subsequent to the administration of progesterone.

Prolongation of the period of injections of progesterone did not increase the effect of the substance on the uterine mucosa but, on the contrary, tended to diminish it; in the course of time long-continued application of this substance led to a refractory state, which in all probability was due to factors other than to the production of immune substances.

20. Loeb, L.: *Am. J. Anat.* **32**:305, 1923; *Australian J. Exper. Biol. & M. Sc.* **9**:141, 1932.

Progesterone stimulated the upper (cylindric) layer of the vaginal epithelium, an effect which could readily be demonstrated in ovariectomized guinea pigs; but under certain conditions it induced mitotic division in these epithelial cells also in nonovariectomized guinea pigs. Rarely, a stimulation was noted in the cuboidal layer of the vaginal epithelium; this occurred especially after long-continued application of progesterone. A combination of estrogen and progesterone led to a still more marked stimulation of the cylindric cell layer than progesterone alone.

Long-continued administration of progesterone produced in some cases a stimulation of the mammary gland, even in ovariectomized animals; however, this effect was irregular and not very pronounced.

It may be concluded that the refractory state of the uterine mucosa of the guinea pig which occurs during the normal sexual cycle if the mechanical stimulation is applied on the tenth day or later after estrus is due not to the exhaustion of the supply of estrogen in the circulation of the animal but either to a diminution in the function of the corpus luteum or to a refractory state which begins to set in at this period of the sexual cycle, or to both of these conditions.

THE PULMONARY APICAL SCAR—AN INQUIRY

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The pulmonary apical scar is quite generally accepted as a healing or healed tuberculous lesion. It is also fairly generally regarded as the final result of a second infection in an allergic but resistant person, as long ago emphasized by Opie.¹ In view of the unique character of this response to the tubercle bacillus it is surprising that so little attention has been given it. Letulle,² it is true, has given a careful histologic description of the lesion under the name *pneumonie ardoisée*, but even this description is not entirely beyond criticism. The genesis of the lesion has almost escaped consideration except perhaps in expressions comparable to that of Letulle, that the lesion is a "sclerosis, always secondary to several fibrocaseous and anthracotic nodules."

For some time I have been interested in these scars and only recently have been over the sections of approximately 50 specimens from persons in whose illness and death tuberculosis played no part, with the rather remote hope that from the scar I might be able to deduce the primary lesion and the sequence of events to its healing. It was realized, however, that even if such a reconstruction of the process were successful it would presumably give evidence only of the character of the allergic reaction in the large proportion of persons who recovered from the infection and not necessarily of that of the reaction in those whose lesion went on to active apical tuberculosis. Thus the study could bear no relation to the controversy as to the supraclavicular or subclavicular origin of adult pulmonary phthisis.

Unfortunately for the purposes of the study, the clientele of the Wisconsin General Hospital is of such character that the scars came from persons of the later decades of life, that is, from the fifth to the ninth decade, with but a single scar from the fourth decade. The causes of death were, in the main, cancer and late cardiovascular disease with, however, a sprinkling of other causes unrelated to tuberculosis. In studying the apical section, the Weigert elastic tissue stain has given the most information when checked with the routine sections stained

From the Pathological Laboratory of the University of Wisconsin.

1. Opie, E. L.: Am. Rev. Tuberc. 6:525, 1922; Tr. Coll. Physicians, Philadelphia, 1927, p. 1.

2. Letulle, M.: Anatomie pathologique, Paris, Masson & Cie, 1931.

with hematoxylin and eosin. The Mallory connective tissue stain has been applied in some cases and is also valuable.

The series of sections was discontinued chiefly because, although there was marked individual variation in superficial extent and in depth of scar, the fundamental features remained constant, and it was felt that little could be added by further lesions in the older persons. All apical indurative lesions are not of the tuberculous type. One may find a silicotic lesion, an organizing pyogenic pneumonic exudate, a blastomatoid overgrowth of smooth muscle in connection with blood or lymph vessels, an angioma, a tumor metastasis with dense stroma and even a Ghon tubercle without a surrounding lesion characteristic of the typical scar.

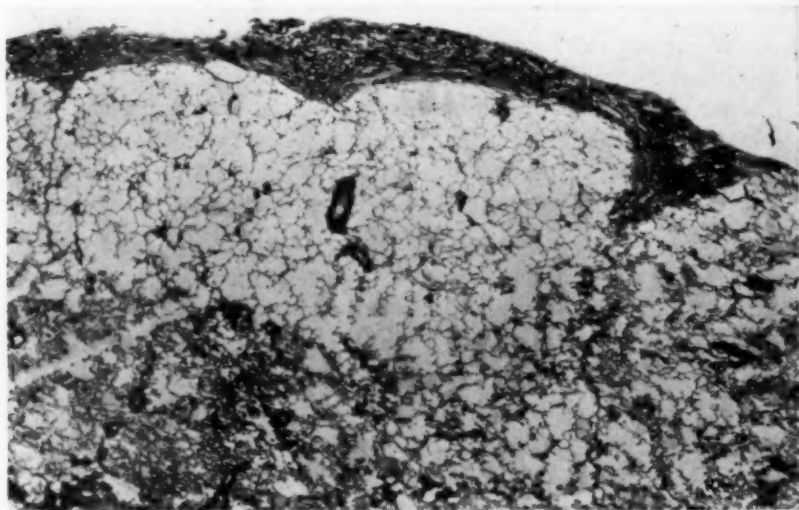


Fig. 1.—Low power view of a superficial scar in a woman aged 53, brought to the hospital in a dying condition from untreated pernicious anemia.

The typical scar has constantly two features: a pleural factor and one involving the immediate subjacent lung parenchyma. Each of these factors may vary in extent and in degree but there is little variation in character.

The pleural lesion may cap a single secondary lobule of the lung or extend widely over many such lobules. Its striking feature is an increase of connective tissue outside of the elastica propria of the membrane. This may be of slight thickness with but a few collagenous strands laid down, or it may be extreme with a heavy layer of parallel fibers of almost tendinous density with slight cellularity. This may give a pearly pleura in the gross picture, resembling the patches of perisplenitis or perihepatitis seen in the so-called iced spleen or liver. This con-

nective tissue is vascularized even when very dense (fig. 3). In most cases there is also an increase in the width of the fibers of the elastica propria with a variable degree of splitting off of new fibers. Fine elastic fibers may also be interspersed among the collagenous fibers.

This vascularized connective tissue layer is apparently the result of organization of a fibrinous exudate at some period. The presence of vascularized fibrous adhesions as seen in a proportion of cases is also indicative of the organization of an adhesive fibrinous pleural exudate,

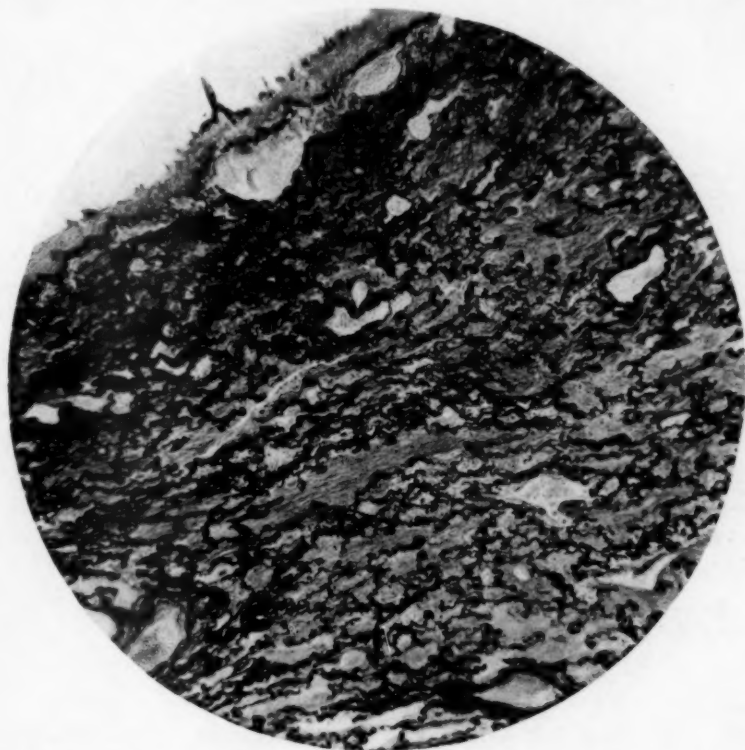


Fig. 2.—Scar from a man of 59 years who died of prostatic hypertrophy and ascending pyelonephritis. Note the normal amount of elastic tissue.

though in regard to the late scars it is not possible to say that the pleural inflammation was contemporaneous with the parenchymal lesion to be described.

The lesion in the underlying substance of the lung like that in the pleura is variable in lateral extent and in this corresponds to the pleural lesion, varying from a few millimeters to several centimeters. It is also variable in depth. It may involve but a millimeter or two of the most superficial part of the lung, or it may extend as a wedge-shaped scar to

a depth of over a centimeter. The shallow lesions have a somewhat scalloped under border, as the lesion extends somewhat deeper along the interlobular septums than in the middle of the secondary lobules (fig. 1).

The essence of the pulmonary lesion is necrosis of the lung framework with obliteration of its blood supply and intra-alveolar organization of exudate. The alveolar and bronchiolar walls are well marked out by the elastic tissue, which is resistant to toxic action and to digestion and persists even in the scars of the most aged persons. With this indication of alveolar limits it is evident that there is a variable degree of

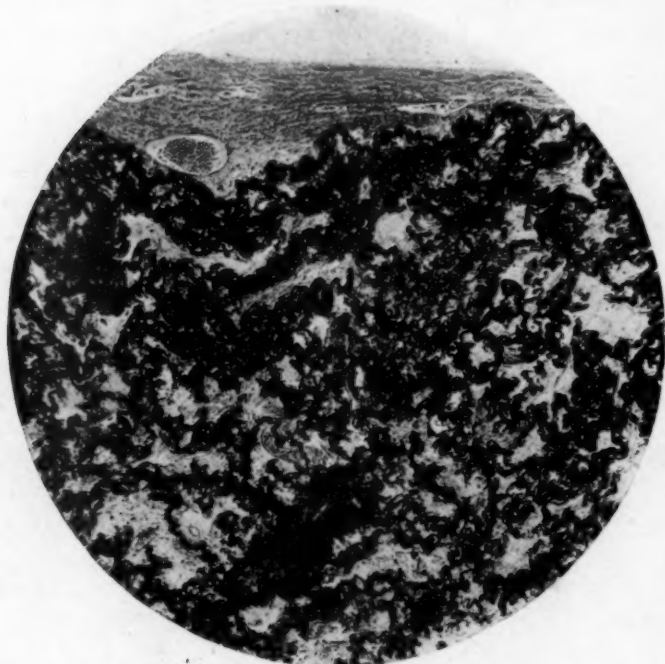


Fig. 3.—Scar from man aged 85 years, with prostatic hypertrophy and terminal acute bronchopneumonia. There is an obvious increase of elastic tissue. All sections stained by Weigert's elastic tissue stain.

collapse of the alveoli but not complete atelectasis. In many specimens and in some areas of most of them some of the alveoli are practically of normal size. The respiratory bronchioles are generally quite widely open. The alveolar and bronchiolar walls are completely devoid of circulation, and all cellular elements have disappeared.

The elastic tissue of the framework presents a variable picture. Ordinarily with partial collapse of the alveoli the tissue has contracted into irregular coils. In many cases this would account for the apparent increase in elastic tissue (fig. 2). In other cases, as emphasized by

Letulle, there is a definite increase in thickness of the fibers and an increase in number of fibers as in the pleura (fig. 3). This increase in number of fibers is apparently due to splitting off of fine fibrils from the original fibers. In some old fibrous scars the elastic tissue appears atrophic and fragmented. However, in some form it is always present.

The lumens of the air sacs and bronchioles are occupied by a vascularized connective tissue. This tissue varies in density and in cellularity, apparently with the age of the scar. In very old scars it may be as dense as the densest pleural scar and almost acellular. In other scars it is looser in structure and often shows, contrary to Letulle's description, a commingling of fine elastic fibers with the strands of collagen. Letulle stated that this organization is identical in character with that of an unresolved exudate due to the etiologic agents of acute pneumonia. This, however, is not the case, as in the latter condition the alveolar walls furnish both the new vessels and the connective tissue cells for the repair process, but in the apex with necrotic avascular walls the new vessels and cells arise from the adjacent living lung substance or from the pleura and grow up into the alveolar spaces. The vessels also are much larger than in the organization of an exudate in acute pneumonia. They tend to run through the center of the alveolar spaces and are often of such size that they occupy most of the alveolar space with but a thin collar of connective tissue about them.

The more common superficial scars which, as stated, involve the peripheral part of the secondary lobules, do not seem to affect the bronchial tree proximal to the respiratory bronchioles. These are not collapsed but appear of normal diameter and, as in the case of the alveoli, are filled with a vascularized connective tissue. The larger bronchioles are involved in the deep scars and may show some irregular hyperplasia.

Lymphoid infiltration is a relatively common feature in these scars. The lymphocytes may occupy a perivascular position in the organizing connective tissue or may infiltrate the pleura above the pulmonary lesion quite generally. In many cases open alveoli in the edge of the scar are completely filled with lymphoid cells. Pigment-bearing phagocytes also migrate into the scar from adjacent bronchioles or alveoli.

Letulle in his descriptions stated that tubercles are always a part of the picture and that such tubercles are surrounded by the apparent exudative process. While one would be justified in denying this only if one had cut serial sections of all scars (and this did not seem a justifiable procedure in this study), I may say that tubercles have been found only in the deep wedge-shaped scars and not in the common superficial scars. Numerous but not serial sections have been cut from those of the latter

type. Some of these lesions have been of such limited extent that a tubercle in any of them could not have been missed.

Below the scars the lung commonly shows compensatory emphysema with endarteritis of the pulmonary vessels.

In considering the problem offered by these apical scars one is impelled to the conclusion stated earlier that the lesion is unique. It does not present the picture of chronic atelectasis or of an organized infarct or of an organization of the exudate of acute pneumonia in delayed resolution. It likewise does not give the picture of the ordinary caseous tuberculous bronchopneumonia with the greatest early effect at the center of the lobule and with the ultimate effect, if healing takes place, of encapsulation but not of organization. One is confronted with a lesion that shows a necrotic avascular lung framework with an organized exudate in the partly open alveoli and bronchioles.

In trying to work backward from so late a lesion as is shown in the scars, one is left only with a number of questions which cannot be answered from the material in hand. In considering the superficial scars, is one to assume that the primary lesion is a sudden necrosis of a sensitized part of the lung on a second infection with the tubercle bacillus? If so, why is the lesion so localized? Does the relation of the lesion to the interlobular septums and the pleura indicate a distribution of the sensitizing bacterial protein through a peripherally flowing lymph stream? Is the apex involved because of sluggishness of flow in this part with greater opportunity for the contained agent to become affixed to lung and pleural tissue? Is relative anemia of the part a factor in such fixation? Is the method of capillary destruction the same as in ordinary caseous tuberculous pneumonia?

If there is sudden necrosis, is there a flooding of the alveoli with a serous exudate containing few cells but with rapid coagulation of fibrin which fails to be digested, becomes hyaline and is eventually organized? This seems plausible in view of the character of the exudate on serous surfaces infected with the tubercle bacillus, where organization and fibrous adhesion often occur. Such involvement of the overlying pleura in these scars would explain the lesions found. Does not this organization exclude caseation of the exudate if one may trust to experience with caseous exudates in other parts of the lung where, as just remarked, encapsulation but not organization ensues?

In the more extensive, deeper, rather wedge-shaped scars with their bases on the pleura must one not assume that the tubercles lying near the apex are older than the scar and responsible for the tissue sensitivity?

These are some of the questions aroused by this study and unanswered by it.

STANDARDIZATION OF TUBERCULIN WITH THE AID OF GUINEA PIGS SENSITIZED BY KILLED TUBERCLE BACILLI IN LIQUID PETROLATUM

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The current laboratory method of standardizing tuberculin is briefly as follows: A group of guinea pigs is infected with virulent tubercle bacilli, and when they are highly sensitive, various dilutions of the unknown and the international standard tuberculin are injected intracutaneously. The reactions are measured one or two days later. The range of tuberculin dilutions used is usually from 1:500 to 1:10,000, and it is essential that well defined but not necrotic reactions should be obtained.¹

It occurred to us that guinea pigs sensitized with heat-killed tubercle bacilli in liquid petrolatum might be substituted for those infected with virulent tubercle bacilli. The possible advantages might be: the elimination of tuberculous animals, the greater uniformity of the sensitizing antigen, repeated use of animals and the convenience of keeping the antigen for a long period of time.

Saenz² found that a large amount of killed tubercle bacilli in 1 cc. of liquid petrolatum injected into the subcutaneous tissue of guinea pigs produces intense sensitization of the skin to tuberculin. The lesions at the sites of injection suppurated. Freund, Casals and Hosmer,³ Casals and Freund,⁴ and Freund, Casals and Genghof⁵ found that repeated small doses of killed tubercle bacilli in liquid petrolatum induce very intense and lasting sensitization without producing discharging

From the Department of Health of the City of New York, Bureau of Laboratories, Antitoxin Laboratory in Otisville, N. Y., and the Michigan Department of Health, Lansing, Mich.

1. Lewis, P. A., and Aronson, J. D.: *Am. Rev. Tuberc.* **7**:404, 1923. Eagleton, A. J., and Baxter, E. M.: *Brit. J. Exper. Path.* **4**:289, 1923.

2. Saenz, A.: *Compt. rend. Soc. de biol.* **120**:870 and 1050, 1935.

3. Freund, J.; Casals, J., and Hosmer, E. P.: *Proc. Soc. Exper. Biol. & Med.* **37**:509, 1937.

4. Casals, J., and Freund, J.: *J. Immunol.* **36**:399, 1939.

5. Freund, J.; Casals, J., and Genghof, D. S.: *J. Immunol.* **38**:67, 1940.

lesions. They observed strong sensitization to tuberculin and active formation of antibodies in guinea pigs eighteen months after the injection of the antigen.

After varying the dosage, we found that the following method of sensitization is satisfactory: Human tubercle bacilli of strain Jamaica 22 are cultured on glycerin agar or Petraghani's medium for approximately three weeks. The growth is removed, placed in a glass container plugged with cotton and kept at 100 C. for half an hour in an Arnold sterilizer. The tubercle bacilli are weighed and suspended in liquid petrolatum by trituration in a mortar so that 1 cc. contains 10 mg. of tubercle bacilli. Two-tenths cubic centimeter of the suspension is injected intramuscularly deeply into each of three sites in the back of the neck of a large guinea pig. The guinea pigs become highly sensitive and suitable for standardization of tuberculins one month after the injection of tubercle bacilli. The sensitization seems to be sustained at a high level for at

Reactions to 0.1 Cc. of a 1:10,000 Dilution of International Standard Tuberculin; Readings Forty-Eight Hours After Injection

Guinea Pig	Weight, Gm.	Diameter of Area of Edema at Given Period Between Sensitizing Injections and Test		
		5 Weeks	7 Weeks	6 Months
1.....	800	10 × 12	10 × 13	10 × 10
2.....	1,065	10 × 12	13 × 13	12 × 14
3.....	870	10 × 10	10 × 11	7 × 8

least six months. Repeating the injections of the antigen promotes sensitization only to a slight degree and seems to be of no particular advantage.

The degree of sensitization is related to the body weight of the guinea pig. It is desirable to use animals weighing more than 800 Gm.

The foregoing protocol illustrates the degree of sensitiveness produced by the method described.

It may be added that guinea pigs can be highly sensitized by a single subcutaneous injection of 10 mg. of BCG. In our experience, animals given such an injection are no more sensitive than those sensitized by killed tubercle bacilli in liquid petrolatum, and in the majority suppurating ulcers develop.

SUMMARY

In standardizing tuberculins, guinea pigs sensitized with killed tubercle bacilli suspended in liquid petrolatum may be substituted for guinea pigs infected with virulent tubercle bacilli.

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THE TEACHING OF EXPERIMENTAL PATHOLOGY

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Experimental methods are now being utilized in courses of general and special pathology in many good medical schools, yet most of those engaged in this teaching seem to know little about methods used elsewhere and about the results obtained with similar methods of teaching in the past. The literature on the teaching of experimental pathology is not cited even by most of those who themselves have written on the subject.

HISTORICAL REVIEW

Osler¹ credited John Hunter with the introduction of experimental pathology. His famous advice to Edward Jenner, a pupil, "Don't think; try," may be cited as a motto of research, in the sense Hunter gave it. Experimental pathology was carried on by Magendi and others, reaching its culmination, according to Osler, in the researches of Claude Bernard.² Knowledge of cardiac disease was revolutionized by the experimental work of Traube, Cohnheim and others (Osler¹). One has only to read the introduction to the "Lectures on Pathology" of Cohnheim³ to know that experimental pathology as a method of research and teaching is not new (Karsner⁴). It is beyond the scope of the present paper, however, to trace the development of experimental pathology from the historical standpoint. The subject gained entry into the curriculum of pathology largely through the work of Cohnheim, Rokitansky and a few of their contemporaries. The reading and the repetition of the experiments of Cohnheim on inflammation and some other subjects³ are fine exercises in pathology even today.

From the Department of Pathology, Cornell University Medical College and New York Hospital.

1. Osler, W.: *The Evolution of Modern Medicine*, New Haven, Conn., Yale University Press, 1922.

2. Bernard, C.: *Leçons de pathologie expérimentale*, Paris, J. B. Baillière et fils, 1872.

3. Cohnheim, J.: *Lectures on General Pathology*, translated from the second German edition by A. B. McKee, London, New Sydenham Society, 1889, vols. 1 and 2.

4. Karsner, H. T.: *Boston M. & S. J.* **167**:531, 1912.

TEACHING OF EXPERIMENTAL PATHOLOGY IN SOME
CONTINENTAL EUROPEAN SCHOOLS

The first institute of experimental pathology was founded by Rokitsansky,⁵ in 1868, in Vienna, and was called Institut für allgemeine und experimentelle Pathologie. Its first director, S. Stricker, introduced the microprojectoscope and the episcope for demonstration to large classes. His lectures were popular, and the audiences were so large (400 students and 200 guests) that he had to lecture twice daily. He was succeeded by Knoll, a physiologist, and the latter, two years later, by Paltauf. Stricker, a physiologist, and his associates were more under the influence of C. Ludwig than of Cohnheim, whose opinions Stricker fought both by the written word and in the lecture room (Basch⁶).

As bacteriology and immunology gained prominence, much of the activity of the institute under Paltauf was occupied with problems in these fields. (A similar trend is to be noted at about the same time in the United States.) In addition, however, the courses in this institute covered, as before, other fields of general and experimental pathology.

One student of this institute, Biedl, became head of a similar institute at the German University of Prague. At this school, bacteriology and immunology were assigned to different departments. Biedl's course of general pathology, as well as that given in Vienna, included large numbers of demonstrations made daily in varied fields of experimental pathology and pathologic physiology. Examinations in the subject were compulsory. The institute at Prague was connected with a "propädeutische Klinik," the functions of which were similar to our correlation courses, but it was managed by the professor of general and experimental pathology, who was thus given an opportunity to conduct studies on patients. This was regarded as an "Arbeitsklinik" for the study of disease processes on patients, with refined and exact methods of physiology.

With the recognition of the usefulness of experimental procedures in the teaching of pathology, something of a revolution took place in this specialty. In most continental schools the subject was split into pathologic anatomy and experimental pathology. These branches were given different chairs, were taught, as a rule, in different buildings and were subjects of separate examinations. The divorce was almost complete, the morphologists studying such gross and microscopic changes as could be learned from autopsy material, while the experimental pathologists

5. Pathology and Related Subjects, in Reprints from Methods and Problems of Medical Education, New York, The Rockefeller Foundation, 1932.

6. von Basch, S.: Beitrag zur Entwicklungsgeschichte der experimentellen Pathologie als Lehrfach, Berlin, Urban & Schwarzenberg, 1905.

investigated the procedures by means of which abnormal changes could be experimentally induced and the genesis and pathologic physiology of disease processes observed. Experimental work was almost banned from many departments of pathologic anatomy.

The smaller continental countries followed the example of the German schools and prescribed in their curriculums compulsory teaching and examination in experimental and general pathology. The activities of several of these institutes can be surveyed in the reprints collected and published by the Rockefeller Foundation.⁵ These show that courses in experimental pathology were appreciated by both students and teachers, and nowhere is there any adverse criticism concerning this type of teaching. The comment of Verzar⁶ in reviewing the activities of his institute in Debrecen, Hungary, expresses, I believe, the opinion of the majority of progressive teachers: "Experimentation is the instrument of inductive research . . . it has become the foundation of our entire medical thinking . . . it controls the correctness of our deductions from experience."

It is my impression, nevertheless, that most teaching of experimental pathology to students in continental schools has been done by means of lectures and demonstrations, except in the realm of immunology and bacteriology. In the latter fields, students themselves had an opportunity to perform test tube experiments. The institutes, however, were open to students interested in assisting in or undertaking experiments in varied fields of medicine.

This review of the course of events in Europe is incomplete. There are several French and German textbooks and handbooks on experimental pathology,⁷ but the description of methods of teaching and the results in different schools at various times are difficult to ascertain.

7. (a) Heinz, R.: *Handbuch der experimentelle Pathologie und Pharmakologie*, Jena, Gustav Fischer, 1906, vols. 1 and 2. (b) Pfeiffer, H.: *Allgemeine und experimentelle Pathologie*, Berlin, Urban & Schwarzenberg, 1924. (c) Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: *Handbuch der normalen und pathologischen Physiologie*, Berlin, Julius Springer, 1927-1932, vols. 1-18. (d) Richet, C., Jr.: *Précis de pathologie expérimentale*, Paris, J. B. Baillière et fils, 1929. (e) Kraus, A. F.: *Grundriss der pathologischen Physiologie und experimentellen Pathologie*, Freiburg, Speyer & Kaerner, 1929. (f) Krehl, L.: *Entstehung, Erkennung und Behandlung innerer Krankheiten: Die Entstehung innerer Krankheiten; pathologische Physiologie*, ed. 13, Leipzig, F. C. W. Vogel, 1930. (g) von Bergmann, G., and Goldner, M.: *Funktionelle Pathologie*, Berlin, Julius Springer, 1932. (h) Franquet, R., and Ginsbourg, B.: *Abrégé de pathologie expérimentale*, Paris, Vigot Frères, 1935. (i) Bürger, M.: *Einführung in die pathologische Physiologie*, ed. 2, Berlin, Julius Springer, 1936.

TEACHING OF EXPERIMENTAL PATHOLOGY IN THE
UNITED STATES

The teaching of experimental pathology in the United States can be traced to William H. Welch and his students. Councilman⁸ expressed well the spirit dominating the school of Welch when he spoke of the desirability of giving students an opportunity to acquire knowledge of the subject by exercise of their own power. Councilman considered no course in pathology adequate which did not include experimental work. "This exercise," he said, "not only teaches students in experimental methods, but also in correlation of cause and effect, and it serves as a living demonstration of altered function."⁸

It has been fortunate for the development of medicine in this country that under the influence of Welch⁹ experimental and pathologic anatomy did not become separated, as evidenced by the wealth of contributions to medicine coming from his students. "To make of experimental pathology a distinct specialty and to endow it with a separate professorship as is done in some foreign universities does not seem to me to be in the direction of the most fruitful and healthy development. Experimental pathology is a handmaid of pathology in all its branches . . ." (Welch⁹).

While on the continent of Europe the teaching of experimental pathology to the student was limited to demonstration to large classes of experiments illustrating some of the more important problems of pathologic physiology, a more practical procedure was tried at the Johns Hopkins Medical School. There MacCallum and Whipple divided the class into small groups of about 8 students, each group working together for a period of from two to three months on one organ or on a group of related organs and utilizing all the methods which experimental pathology could bring to bear on the problems under consideration (Pearce¹⁰).

The first comprehensive description of the teaching of experimental pathology at Baltimore appeared in a series of articles by MacCallum.¹¹ An article by Rous¹² followed, describing the course in Michigan, then one by Pearce¹⁰ describing the course at Philadelphia, while Councilman⁸ and Karsner⁴ published their method as practiced in Boston.

Teaching at the Johns Hopkins University.—MacCallum¹¹ began his course at the Johns Hopkins Medical School in 1905. Describing this, he stated:

8. Councilman, W. T.: *Pathology: A Manual for Students and Teachers*. Boston, W. M. Leonard, 1912.

9. Welch, W. H.: *Bull. Johns Hopkins Hosp.* **1**:25, 1890.

10. Pearce, R. M.: *Bull. Johns Hopkins Hosp.* **22**:403, 1912.

11. MacCallum, W. G.: *Bull. Johns Hopkins Hosp.* (a) **17**:251, 1906; (b) **18**:327, 1907; (c) **19**:215, 1908.

12. Rous, P.: *Bull. Johns Hopkins Hosp.* **19**:335, 1908.

"It seemed desirable to teach pathology, not merely from the morphological standpoint, but more particularly to bring clearly before the student the alterations in function which result from disease by applying to the organs in the course of the reaction to experimentally produced lesions all of the exact methods of physiology. By this means, always in association with anatomical studies, it is possible to correlate in the mind of the student much more clearly than otherwise the changes in function and the anatomical alterations."

It was planned to produce experimentally the lesions commonly met with and to use uncomplicated lesions for illustration of the principles involved. This course was conceived as an adjunct to clinical medicine, providing greater opportunities for studying the patients.

Facilities for this work were afforded in the Hunterian Laboratory for Experimental Medicine. The course in the first year was devoted entirely to the circulatory system. It lasted about two months, with three afternoon exercises a week. In a general way, the lectures of Cohnheim formed a model for the course, some of the experiments described by him actually being carried out.

In connection with the course, time was allotted for the study of gross and microscopic anatomy of the lesions to be studied experimentally. The experiments performed included the production of insufficiency and stenosis of the various valves, with recordings of the pulsations and pressure changes in different parts of the circulatory system before and after the production of the lesion. The effects of pericarditis were simulated by the introduction of fluid under pressure into the pericardium. These experiments and their results have been fully described by MacCallum.¹¹

The second division of the course, described in 1907,^{11b} was concerned with diseases of the organs of internal secretion. The organs studied include the thyroid, adrenals, pancreas, parathyroids and carotid bodies. The third course was concerned with the organs of respiration.^{11c} It was proposed to study the digestive tract in the next year.^{11e}

In subsequent years the length of time devoted to teaching experimental pathology at the Johns Hopkins Medical School was greatly decreased. In a more recent article,⁵ MacCallum wrote: "In former years courses in the experimental study of functional disturbances produced by disease have been given to select groups of students and have proved to be extremely profitable, not only because they teach experimental methods, but because they evoke an inquiring attitude which stimulates many students to proceed with investigations of problems that arise. Such a course has been arranged for, and it is confidently hoped that the plan may be carried through."

Evidently facilities were not as adequate in most places for class experimentation as in Welch's laboratory, but it also seems evident from the numerous articles quoted that this type of teaching itself was in the experimental stage with constant urge to improve and adapt it to changing conditions. There seems no doubt, however, that pathologists who were students under Welch give prominence in their courses to facts found on experimental evidence. MacCallum's textbook itself¹³ is an admirable review of experimental pathology, even though scheduled teaching utilizing experimental methods has declined in his school.

13. MacCallum, W. G.: *A Textbook of Pathology*, ed. 6, Philadelphia, W. B. Saunders Company, 1936.

Teaching at the University of Michigan.—Peyton Rous, at nearly the same time as MacCallum, offered to junior medical students at the University of Michigan an elective course in "physiological pathology."¹² It extended over a period of three weeks, with a three hour session each day, and was given twice a year. The teaching was concerned mainly with disturbances of circulation and respiration, important clinically. Experiments and conferences on the topics illustrated by the experiments, with the study of museum specimens, made up the major part of the work. Numerous experiments concerned with disturbances of the cardiovascular system and respiration are described in detail. "The value of the work in its present limited scope must be measured less by the amount of ground covered than by the point of view given to the students for the study of disease at the bedside."

Teaching at Harvard Medical School.—Councilman's textbook of pathology⁸ contains a description by Karsner of numerous experiments carried out at Harvard Medical School by groups of students working under supervision. Of these experiments, the following ones are listed in Councilman's book:

Concerning degeneration and inflammation: Ligation of a renal artery; chloroform poisoning; ether necrosis; specific immune serum necrosis. (p. 45).

Concerning inflammation and repair: Inflammation of the rabbit's ear produced by heat and croton oil; the same after cutting the cervical sympathetic nerves; pleurisy produced by aleuronat and Staphylococcus; abscess produced by Staphylococcus or by the colon bacillus; embolic abscesses produced by Staphylococcus; phagocytosis of liquid petrolatum and of colon bacilli; opsonic effects; wound healing; regeneration of liver and of cornea. (p. 58).

Concerning diseases of the blood: A study of living leukocytes in drops of human blood; hemolytic anemia produced by rattlesnake venom; hematuria produced by cantharidin; hemolytic jaundice produced by specific immune serum, with phagocytosis of destroyed blood cells; focal necrosis in the liver and nephrosis; testing of fragility of erythrocytes by means of salt solution; the role of complement in hemolysis. (p. 69).

Concerning diseases of the circulatory system: Thrombosis of veins produced by trauma and heat; embolism produced by tobacco seeds, olive oil and air; pericarditis produced by olive oil, with kymographic tracing; aortic stenosis resulting from constriction of the aorta, with observation of heart action and of blood pressure; aortic regurgitation produced by injury of aortic leaflets, with effect on pulse and blood pressure and observations with the stethoscope; myocardial degeneration produced by injection of alcohol in the cardiac muscle, with kymographic registration of heart action; arteriosclerosis produced by epinephrine. (p. 82).

Concerning infection: Effect of diphtheria toxin in guinea pigs; protection by antitoxin serums; experimental tuberculosis and syphilis; pneumococcus pneumonia. (p. 334).

Concerning diseases of the kidney: Experimental hydronephrosis produced by ligation of a ureter; pyonephrosis produced by injection of colon bacilli into an obstructed ureter; renal damage by potassium chromate, cantharidin, mercury bichloride, uranium nitrate and potassium arsenate, with registration of kidney volume, blood pressure and diuresis. (p. 363).

Concerning diseases of the alimentary canal: Acute toxic gastritis produced by phenol and other acids; hemorrhagic erosion of the stomach produced by snake venom and by agar injected beneath the mucosa; surgical intussusception. (p. 370).

Concerning diseases of the pancreas: Acute hemorrhagic pancreatitis and fat necrosis resulting from injection of bile into the pancreatic duct; diabetes following extirpation of pancreas; puncture glycosuria. (p. 377).

Concerning diseases of the liver: Jaundice produced by ligation of common duct; obstructive cirrhosis. (p. 389).

The scheme as outlined by Karsner¹⁴ operated successfully for three years at Harvard Medical School, but in later years under Councilman and under his successor, Wolbach, class experiments were not included in the regular course of general and special pathology.

Subsequently Karsner¹⁵ utilized this type of teaching in association with F. W. Peabody in the clinicopathologic conferences held for students. These exercises were concerned mainly with aortic valvular disease, arteriosclerosis, diabetes mellitus and nephritis. Experimental and human data and material were presented, and patients with the corresponding disease were demonstrated. Thus anatomic, physiologic and clinical aspects of the disease were correlated.

Teaching at the University of Pennsylvania.—Brief special courses in experimental pathology had been given at the University of Pennsylvania by Leo Loeb¹⁶ during the first decade of this century. This course was greatly extended by Pearce¹⁰ to include the entire class.

A class of 90 men was divided by Pearce into two sections, each meeting for an hour once a week during a period of fourteen weeks. At each session at least five demonstrations were made simultaneously under the direction of a member of the department. Twelve minutes were given for each demonstration. A mimeographed sheet was given to each member of the class two days before the demonstration. The following are some of the experiments demonstrated by Pearce and his assistants (Eisenbrey, Karsner, Veeder, Austin) in this course:

Concerning inflammation and repair: Rabbit's ear exposed to hot water or rubbed with croton oil; pleurisy produced by aleuronate or turpentine; embolic abscesses produced by *Staphylococcus*; healing of aseptic wounds; healing of infected wounds.

Concerning degeneration and necrosis: Cloudy swelling produced by cantharidin; fatty degeneration produced by phosphorus; necrosis following ligation of the superior mesenteric and renal arteries; gas gangrene produced by *Bacillus aerogenes capsulatus*; caseation in experimental tuberculosis of the guinea pig; thrombosis, embolism and infarction.

These and other experiments on blood destruction and jaundice, on induced changes in the heart, lung, liver, pancreas, stomach, kidney and ductless glands and on hypersusceptibility and immunity are fully described by Pearce.¹⁰

14. Karsner.⁴ Councilman.⁸

15. Karsner, H. T.: Boston M. & S. J. **170**:723, 1914.

16. Loeb, cited by Karsner.⁴

Unusually successful were the experiments given by Pearce in conjunction with W. T. Longcope in experimental pathology and physical diagnosis. These experiments served to bridge the gap between pathologic anatomy and clinical observation.

The teaching of experimental pathology at the University of Pennsylvania as practiced in recent years has been outlined in a book by Wagoner and Custer.¹⁷ The teaching methods follow closely those of Karsner.⁴

Surgical technics and other laboratory procedures and the general care of the animals are described in detail, and normal values (such as blood cell counts and those of blood chemistry) for laboratory animals are listed. The exercises described cover both general and experimental pathology. This book is useful as a guide for class demonstration of what are now considered classic experiments.

More recently this teaching has been supplanted in part in this school by exercises similar in type to those conducted at Cornell, based on significant experimental observations appearing in current literature.

Teaching at Western Reserve University.—Karsner, a pioneer in teaching experimental physiology and pathology,¹⁸ has maintained interest in this subject for a period of three decades. At the Institute of Pathology, Cleveland, organized and headed by him,⁵ experiments are performed annually in connection with the study of the circulatory system, respiratory system and kidney.

If time permits, experiments dealing with the lungs, liver, pancreas and alimentary canal are also made. During the periods when circulation is being studied, groups of 5 students work in the experiment room under the direction of the professor or an associate professor. Each group studies with a kymograph the effects of such conditions as hydropericardium, aortic stenosis, aortic insufficiency and experimental myocardial degeneration, infarction and air embolism. With the multiple stethoscope all students hear the murmur of aortic stenosis. A quiz conference at the end of the period includes the demonstration of the physiologic results obtained by the small groups. The experiments on the respiratory tract deal with the effects of hydrothorax, acute asthma, anaphylactic shock, and acute bronchitis from irritant gases, with auscultatory demonstration of rales and experimental pneumonia. The physiologic work in renal disease is concerned principally with the production of experimental nephrosis and the study of the accumulation of nitrogenous products and the excretion of phenosulfonphthalein. A correlation period follows.

Teaching at the University of Chicago.—An elective course has been given at the University of Chicago by Cannon on the defense mechanism of the body. In this course are considered systematically the various structures and forces available to the animal body for defense against disease (Wells⁵). Laboratory work on the principles and methods of immunology is also given. Approximately one third of the

17. Wagoner, G., and Custer, R.: *A Handbook of Experimental Pathology*, Springfield, Ill., Charles C. Thomas, Publisher, 1932.

18. Karsner.⁴ Councilman.⁸ Pearce.¹⁰

class took the course, but only about 16 took the laboratory work. The experiments were performed in small groups. Although the work was elective, students participating received credit.

The course consisted of two lectures a week for a period of approximately eleven weeks with laboratory work and the study of approximately two hundred slides, given to illustrate the problems studied. The following are examples of the experiments:

A study was made of the blood clearance of particulate matter (bacteria or dye injected intravenously). In this work blood cultures were taken, and the animals were killed at various intervals and the organs cultured and sectioned. The degrees of resistance offered to micro-organisms by different surfaces were compared. Other studies were concerned with lipid pneumonia—a demonstration of the hazard of nasal antiseptics; peptic esophagitis; protective reactions in lymphoid tissues and the spleen; hormonal alterations in the character of the vaginal epithelium in relation to resistance offered by the epithelium; the Schwartzman phenomenon; the Duran-Reynolds effect, and agranulocytosis produced by drugs.

The course was highly successful. The experiments themselves were not original enough for publication, but they were most instructive and were often continued by the students, giving them a thorough training in experimental methods, as indicated by the number of original investigations published by those who participated in this course. It is unfortunate that Cannon, who has since succeeded Wells as head of the department, has not been able to schedule a practical elective course of this type.

Teaching at Washington University, St. Louis.—Compulsory participation in class experiments in the regular course of general and special pathology was introduced at St. Louis by Moore.¹⁹ For this purpose, a class is divided into groups of 4 students. Experiments are so selected as to illustrate the causes, genesis and natural course of disease. These experiments are of long duration in contrast with those of Pearce and others, which are terminated within a day or two. At the beginning of the course the experiments are set up; during the course observations are made, and at the end of the course the progress made is reported and discussed. For example, tumors are produced by virus, and the growth occurring is observed and studied histologically. Tumors thus produced by the students are again transmitted by cell-free material prepared from the original tumor. In another experiment, tumors are transmitted to irradiated and nonirradiated animals, and the effect of roentgen rays on the susceptibility of the host to the neoplasm is studied and correlated with other changes produced by the roentgen rays.

Teaching at Cornell University Medical College.—The teaching of experimental pathology as organized by Dr. E. L. Opie has been part of the regular course of general and special pathology since 1932. The class is divided into groups of from 3 to 5 students. At the beginning

19. Personal communication to the author, 1941.

of the course a list of problems is posted, and students are asked to express their first, second and third choice. The experiments are carried out during the course of pathology under the direction of an instructor at times arranged by appointment, and at the end of the course a paper on the literature of the subject and a description of the experimental methods used, together with the results and conclusions, are presented before the entire class and discussed by the students. A period of fifteen minutes is given to the presentation of the paper; this is followed by an open discussion lasting for fifteen minutes. The discussion is closed by the sponsor of the experiment.

The amount of time devoted to this work varies greatly with the character of the experiments and the students' interests. Those who showed no interest in the experimental work were urged to attend; others use part of their free afternoons to carry out the work of their choice.

Instructors are directed to emphasize to students the importance of the following procedures: Careful records are to be kept, and pertinent details should be noted throughout the progress of the experiment; breed, sex and weight, as well as other data concerning the animals used, should be registered, and the students should assume responsibility for the experimental animals. Technical methods, such as blood counting, should be practiced before they are applied to the experiment. Careful and detailed autopsies must be made on all animals that die, and records kept. Students should be oriented in relation to the literature of the subject of the experiment. A written report, to be handed in after completion of the experiment, should include (1) a general discussion of the subject of their study with a consideration of the related literature, (2) a description of their experiment and a discussion of the results they have obtained, and (3) a bibliography containing a full list of all authors referred to in the paper, with title of paper, place of publication, year, volume and page.

Participation in class experiments was at first compulsory; later approximately one fourth of the class was excused. Although students who had difficulties in the preceding year in any subject were advised informally not to sign up, nevertheless many expressed their disappointment and were therefore admitted to the course.

The present procedure developed gradually by trial. The instructors were asked to propose experiments illustrating some fundamental aspects of pathology in which they themselves were interested. The proposal had to be based on some well founded observation, which was repeated and complemented by experiments aimed to test the validity of additional original ideas. These were sifted by Dr. Opie and, together with experiments proposed by himself, were posted and carried out as already described. Thus many class experiments resulted in original contributions which were either brought to completion and published by the students, with the aid of instructors, or completed by the instructors themselves. The following are examples of such class experiments:

1. On inflammation: Class experiment: The technic of observing the vascular changes of inflammation in the exposed mesentery of the frog and comparison of

the action of the inflammatory irritants used. Paper: "The Influence of Inflammation on the Absorption of Substances of Varied Diffusibility," published in the *Journal of Experimental Medicine* (67:619, 1938).

Class experiment: The embryologic development of inflammation. Paper: "Inflammation in Embryonic Life," to be published.

Class experiment: The genesis of a virus infection producing encephalomyelitis in mice and its relation to poliomyelitis in man. Paper: "A Strain of Virus Producing Meningo-Encephalitis in Mice with Special Reference to Pathogenesis," published in the *Journal of Infectious Diseases* (69:232, 1941).

Class experiment: The effect of irradiation on the course of streptococcal and staphylococcal infections in the skin of rabbits. Paper: "The Effect of Roentgen Therapy upon Infections Produced in the Skin of Rabbits with Cultures of Streptococcus Hemolyticus and Staphylococcus Aureus," published in the *American Journal of Roentgenology and Radium Therapy* (46:96, 1941).

2. On transplantation and regeneration: Class experiments: The effect of hormones on prostatic tissue transplants in the anterior chambers of the eyes of rabbits. The effect of the administration of drugs affecting the sympathetic and the parasympathetic nervous system on vesicular tissue transplantation in eyes. The physiologic response of vesicular transplants in the eyes of female rabbits to injections of hormones. Papers: "The Physiological Response of Prostatic and Vesicular Transplants in the Anterior Chamber of the Eye," published in the *Journal of Experimental Medicine* (66:281, 1937); "Variation in the Size of Transplants of the Prostate and Seminal Vesicle in the Anterior Chamber of the Eye," published in the *Journal of Experimental Medicine* (66:273, 1937); "The Physiological Response of Ocular Transplants of the Seminal Vesicle in Female Rabbits," published in *Endocrinology* (23:468, 1938).

Class experiment: The regeneration of liver in young and old rats and the metabolism of regenerating liver tissue. Paper: "Regeneration of the Rat's Liver at Different Ages. Metabolism of the Embryonic, the Neonatal and the Regenerating Rat Liver," published in this issue of the ARCHIVES OF PATHOLOGY.

3. On intercellular substances: Class experiment: Organic chemical analysis of protein matrices which undergo calcification. Papers: "Studies on Cartilage: I. Some Effects of Mediums of Different p_H Values on the Composition of Cartilage," published in the ARCHIVES OF PATHOLOGY (33:145, 1942). "Studies on Cartilage: II. A New Histochemical Reaction with High Specificity for Cartilage Cells," published in the ARCHIVES OF PATHOLOGY (33:174, 1942).

4. Tuberculosis: Class experiments: The histology of lesions produced by heat-killed tubercle bacilli and paraffin oil simultaneously injected into the skin of rabbits. The absorption of heat-killed tubercle bacilli from the gastrointestinal tract in the presence of oil. Paper: "Sensitization and Antibody Formation After Injection of Tubercle Bacilli and Paraffin Oil," published in the *Proceedings of the Society of Experimental Biology and Medicine* (37:509, 1937).

Class experiment: Experimental tuberculosis in a resistant animal. Paper: "Sensitization, Antibody Formation and Lesions Produced by Tubercle Bacilli in the Albino Rat," published in the ARCHIVES OF PATHOLOGY (27:289, 1939).

5. Blood-forming organs: Class experiment: Tissue culture studies on the relation of monocytes to endothelial cells and fibroblasts. Paper: "Cultural Studies on the Relationship of Lymphocytes to Monocytes and Fibroblasts," published in the ARCHIVES OF PATHOLOGY (25:46, 1938).

Class experiment: Proteolytic enzymes in the spleen and their relation to acute splenic tumor. Paper: "Proteolytic Digestion of Red and White Blood Corpuscles," published in the *American Journal of Pathology* (18:333, 1942).

Class experiment: The relation of prothrombin to bone marrow activity. Paper: "Prothrombin Formation Following Irradiation of Bone Marrow by Roentgen Rays," published in the *American Journal of Roentgenology and Radium Therapy* (**46**:356, 1941).

6. On tumors: Class experiments: The genesis and transmission of adenoma of the lung in mice. The changes produced by environmental factors in transmissible adenomas of mice. Paper: "The Character of Changes Occurring in the Course of Transplantation of Two Strains of Lung Tumors in Mice," published in *Cancer Research* (**2**:116, 1942).

Class experiment: The characteristics of tumors of the prostate produced with 1,2-benzpyrene in the white rat. Paper: "Production of Tumors of the Prostate of the White Rat with 1-2 Benzpyrene," published in the *American Journal of Cancer* (**30**:731, 1937).

Class experiment: The phosphatase content of malignant osteoblasts of chicks and its relation to bone formation. Paper: "A Histological Study of the Distribution of Alkaline Phosphatase in Various Normal and Neoplastic Tissues," published in the *American Journal of Pathology* (**17**:303, 1941).

Class experiment: Search for virus in chicken tumors produced by benzpyrene. Paper: "Attempts to Propagate Fowl Tumors Produced by Benzpyrene and by Virus," published in the *ARCHIVES OF PATHOLOGY* (**28**:212, 1939).

Class experiment: Transmission of the chicken sarcoma produced by methylcholanthrene and the nature of the transmitting agent. Paper: "Further Investigation on the Transmission of Induced Tumors in Fowls," published in *Cancer Research* (**1**:609, 1941).

7. On leukemia: Class experiment. Identification of the malignant cells and study of the genesis of a transmissible strain of chloroleukemia of mice. Paper: "The Transmission of Chloroleukemia of Mice," published in the *American Journal of Pathology* (**14**:217, 1938).

Class experiment: Genesis of leukemia produced in mice by percutaneous injection of methylcholanthrene. Paper: "Production of Leukemia in Mice by Methylcholanthrene and X-Rays," to be published in the *Journal of the National Cancer Institute*.

Class experiment: Experimental production of myeloid metaplasia in mice. Paper: "Myeloid Leukemia and Non-Malignant Extramedullary Myelopoiesis in Mice," published in the *American Journal of Cancer* (**37**:1, 1939).

Class experiment: The histologic character of a splenic tumor of mice and its transmission. Paper: "Splenic Reticulum Cell Tumors in Mice," published in the *American Journal of Pathology* (**16**:449, 1940).

This list is incomplete. The studies described in the papers of Moore, Hellmann and Jacobius and that of Saxton published in this issue of the *ARCHIVES OF PATHOLOGY* were likewise subjects of class experiments. The results of several recent experiments will be published. Dr. Opie's support throughout the experiments and in the preparation of the papers was generous and of immense benefit.

The subjects covered are broader than the titles given would indicate and include several problems related to the following topics: intercellular matrices; cytologic changes resulting from ischemia and other damage;

vitamin deficiencies; formation of bile pigment *in vitro*; capillary fragility in jaundice; experimental diabetes with dextrose tolerance tests; roentgen ray changes; effects of splenectomy; phagocytosis and opsonic effects; recovery from experimental cirrhosis; nephrosis produced by different agents. Facilities were favorable for the study of tuberculosis, and several experiments were performed annually to study various aspects of experimental tuberculosis. These include the genesis of tuberculous infections, the biologic reaction produced by constituents of tubercle bacilli, enzymes in tuberculous tissues, sensitization and desensitization in relation to tuberculosis (*in vitro* and *in vivo* experiments), the relation of hypersensitivity to immunity, and experimental tuberculosis of the adrenals and of other organs.

COMMENT

The utilization of experimental methods in teaching pathology has undergone many changes since students of Welch introduced it in this country. Bacteriology and immunology became independent subjects, taught, as a rule, in different departments. Many of the experiments performed in the classes of Pearce and Councilman and others became incorporated in regular courses of bacteriology and immunology. Others are now taught in courses of applied pathology, which at Cornell University is part of the department of medicine. Many experiments taught in the special course of Pearce and Councilman and others became routine procedures, e. g., the testing of the fragility of erythrocytes by means of salt solutions of varying tonicities. Microscopic sections showing changes occurring spontaneously or induced experimentally are now loaned to students, who can study them leisurely. Good human museum and hand specimens are now available to demonstrate the gross changes which in earlier years were produced by students experimentally in class exercises—e. g., gastritis due to phenol—and it no longer seems worth while to perform such an experiment. However, when neither human nor experimental material is available for demonstration to students, class experiments may serve the purpose of securing such material. The teaching of experimental methods as was practiced several decades ago has survived its usefulness, and it has been abandoned in most of the great schools in which it originated. It is opportune, therefore, to inquire about the methods which suit present conditions and are a useful supplement to the regular course of general and special pathology.

It is fortunate for the development of teaching experimental pathology that facilities for experimental work are, in general, better now than they were in the past. Most good institutions receive an increasing amount of financial support from foundations and from individuals for certain fundamental investigations and employ full time or part time

research workers. Participation of students in some research projects thus supported may be of benefit to the projects studied and the students alike.

In planning class experiments it should be first considered that every course of pathology must cover an "irreducible minimum" of general and special pathology necessary to build a foundation for the clinical branches of medicine with the primary aim to train physicians for the care of the sick. This can be well accomplished by a critical consideration in the lecture of knowledge built on observation and experimentation. But a large percentage of the students are able to receive a superior training by learning through experimental methods the evaluation of observations from experience of their own. The success of experimental courses in pathology given during the recent decades in several schools, the numerous published studies which have their origin in class experiments and the list of many brilliant teachers and investigators whose paths were marked out in such courses should lend encouragement to this type of teaching.

A program suitable for all schools cannot be given. The broader aims of this teaching are still the same as those expressed by MacCallum, Rous, Pearce, Councilman, Karsner and others, but different methods are needed to carry them out. The scope of the class experiments must be limited by the relative number of the teaching staff, their interest and ability, by the quality of the student body and the time allotted in the curriculum to pathology and related subjects, and by the facilities of the department. Courses made highly successful in one institution by the specialized interest and ability of one teacher may fail elsewhere; e. g., the course of Longcope and Pearce on the production of uncomplicated cardiac lesions, with physical diagnosis, or that of Cannon on the defense mechanism of the body could not be duplicated by an average teacher. Repetition of the overwhelming number of class experiments formerly recommended is no longer profitable because most of those experiments served to demonstrate physiologic methods, bacteriologic and immunologic phenomena, and changes in blood, most of which are now adequately taught in the corresponding special subjects. Most simple experiments, the outcome of which is known and can be readily demonstrated, often with human material, are unattractive.

The simplest form of learning the lessons of experimentation can be attained by including in the class set sections of lesions produced experimentally, e. g., sections of organs of mice that had received intravenous injections of carbon particles. The students may study these sections at leisure and describe the relative concentrations of carbon in different organs and in the cells which stored the particles. Similarly, gross specimens can be made available which show changes produced

experimentally and their genesis, e. g., primary tuberculous infection as compared with reinfection made under comparable conditions. Similar experimental and human materials can be compared and used as the nucleus of a colloquium for broad underlying principles, e. g., the role of immunity in modifying the defense reaction of the body. Indeed, "numberless pathological lesions are real experiments by which physicians can profit."²⁰

Demonstration or repetition by the students of a few well chosen classic experiments may be helpful to emphasize some fundamental principles or to demonstrate newer methods of experimental approach with broad potentialities—e. g., the grafting of tissues into the anterior chamber, the utilization of the chorioallantois membrane of the chick embryo and the use of the transparent chamber of the rabbit's ear. Motion pictures have not taken the place which they deserve in the demonstration of pathologic processes in living objects—e. g., the locomotion of different leukocytes, phagocytosis, normal and pathologic mitoses, inflammation and the formation of granulation tissue.

Compulsory class experiments as part of the regular course of pathology have not been proved satisfactory or feasible, with the possible exception of the course given at Western Reserve University, but a large part of the student body profits considerably from participation in class experiments. Highly successful courses have been conducted in this field by the assisting teaching staff, as electives. These may take the place of class experiments.

Physiologic experiments illustrating acute disturbances, which formed a large part of experimental courses in pathology in former years, have been made superfluous by class experiments now offered in courses in physiology and pharmacology.

By far the best are experiments which demonstrate the causation, the genesis and the pathologic physiology of disease and which enable the student to observe and to explain the significant changes. These experiments require, as a rule, observation over longer periods. Their nucleus should be a sound classic experiment or one currently reported. The use of the methods of the exact sciences and consultation with specialists should be encouraged. Numerous examples of this type of class experiment have been given, but instructors should look for problems in the current literature or in the autopsy material or in the clinic. The procedure of consulting the literature, evaluating the papers read, describing the study in the form of a paper, presenting the paper and discussing it before the class may all be part of this exercise.

Animal experiments are one of the best means of experimental pathology, but functional, histologic, chemical and other methods applied to

20. Bernard, C.: *Introduction to the Study of Experimental Medicine*, translated by H. C. Greene, New York, The Macmillan Company, 1927.

pathologic tissues available at autopsy may be equally advantageous in class experiments. As examples, the studies of elasticity of vessels in relation to age and the histochemical demonstration of phosphatases in different tissues have already been mentioned. A study of the vascular pattern of diseased human hearts by injection of radio-opaque material as described by Schlesinger²¹ or of changes in rate of flow by perfusion of organs (Cox and Dock²²), the demonstration of the pattern of diseased nephrons (Oliver and associates²³) and the composition of intercellular matrices (Hass²⁴) are only a few of the many possible lines of investigation with human material suitable for special class exercises.

Unless taken up and carried out with interest and enthusiasm, such a course is bound to fail. Zeal and enthusiasm, however, should not result in neglect of the regular course, and the instructor handling a small experimental group may act as guide and tutor for the group. Lack of participation in the course should not be held against students, but credits should be given for good qualities exhibited in the special course.

The impression is gained that the experimental course has drifted from more practical to more "academic" problems. Were this true, it would not degrade pathology. Long ago Welch, one of the most successful teachers of this subject, advocated treating pathology as a branch of the natural sciences which "in and for itself alone deserves to be studied."⁹ But are not "academic" problems often the source of great practical discoveries? Did not studies on the regeneration of hemoglobin lead to the control of pernicious anemia? Students, according to current curriculums, can actively participate in only a few experiments; therefore, the course of pathology can do no more than show students methods which "can teach us to develop and use to better purpose the faculties with which nature has endowed us . . ." " . . . a good method promotes scientific development and forewarns against those numberless sources of error which they meet in the search for truth; this is the only possible object of the experimental method."²⁵ Claude Bernard devoted a whole course at the Collège de France to the study of *curare*, "not for the sake of the substance itself, but because this study shows how the simplest single determinism . . ., re-echoing successfully from all other vital units, leads to secondary determinisms which grow more and more complicated till death ensues."²⁶

21. Schlesinger, M. J.: *Arch. Path.* **30**:403, 1940.

22. (a) Cox, A. J., and Dock, W.: *J. Exper. Med.* **74**:167, 1941. (b) Dock, W.: *ibid.* **74**:177, 1941.

23. Oliver, J., and Luey, A. S.: *Arch. Path.* **19**:1, 1935.

24. Hass, G.: *Arch. Path.* **30**:240, 1940. Hass, G., and Schultz, R. Z.: *ibid.* **27**:334 and 583, 1939.

25. Bernard,²⁰ p. 35.

26. Bernard,²⁰ p. 88.

SUMMARY

The courses in experimental pathology given in different schools during the past decade have varied greatly in character, but most of the teachers who have conducted them agree that they are a complement to the regular course and should be offered only to the better students. There are many ways of utilizing experimental methods in teaching pathology, and instructors organizing such a course will profit from a study of past experience with this method of teaching. The character of the exercises must be adapted to existing local conditions. There are the best of reasons why they should be part of the curriculum. Experimental pathology discloses to students the dynamic quality of pathologic events, makes them aware of action and reaction within the diseased body, sharpens their reasoning, provides them through actual experience with the ability to evaluate lectures, readings and observations, and shows them how to formulate and test working hypotheses of their own.

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STUDIES OF AMYLOID

II. THE ISOLATION OF A POLYSACCHARIDE FROM AMYLOID-BEARING TISSUES

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This report is concerned with the isolation of a polysaccharide from amyloid-bearing spleens and livers obtained post mortem from patients with chronic pulmonary tuberculosis. The amount and the morphologic characteristics of the deposits of amyloid were defined by histologic methods and correlated with the yields of the polysaccharide. Certain properties and derivatives of the product were studied quantitatively and compared with those of chondroitin-sulfuric acid obtained from infantile epiphyseal cartilage.

MATERIAL AND METHODS

Material.—Nine livers, six spleens and thirty femoral epiphyseal cartilages were available for study. The cartilages were obtained post mortem from newborn infants. All livers and spleens were obtained post mortem from adult patients with advanced chronic pulmonary tuberculosis. Six livers, weighing 1,800 to 3,500 Gm., and three spleens, weighing 300 to 450 Gm., contained amyloid deposits. In two livers and one spleen, these deposits had a pink color, which was attributable to congo red that had been injected intravenously for diagnostic purposes during life. Three livers, weighing 1,200 to 1,600 Gm., and three spleens, weighing 185 to 225 Gm., contained no amyloid deposits which could be demonstrated by histologic methods.

Morphologic Methods.—A microscopic study of each liver and each spleen was made. Fresh blocks of tissue and material preserved in solution of formaldehyde U. S. P. were available in each instance. Fresh tissue was cut into sections with a freezing microtome set at 20 to 25 microns. One group of sections was stained with 1 per cent compound solution of iodine (Lugol's solution), a second group with aqueous 0.01 per cent crystal violet and a third group with aqueous 2 per cent congo red. A fourth set of fresh sections was examined with a polarizing microscope. All sections were placed on glass slides, counterstained with aqueous hematoxylin and mounted in water under a cover slip preparatory to microscopic study.

The tissues preserved in solution of formaldehyde were embedded in paraffin. The permanent sections were stained with hematoxylin and eosin and with phosphotungstic acid-hematoxylin by Mallory's method.

Methods of Extraction and Purification of Products.—After resection of blocks of fresh tissue for the purposes which have been discussed, all remaining tissue

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from each liver and spleen and all epiphyseal cartilages were divided into small pieces and washed at 10 C. for twenty-four hours with successive changes of water. Dehydration of the tissues and removal of lipids were completed by successive extraction with several volumes of 95 per cent alcohol and finally with ethyl ether. Following extraction with ether, the tissues were dried at 50 C. for twenty-four hours and then ground in a machine to a fine powder.

This material was available for subsequent extractions by two methods. In one method the solvent was aqueous half-normal sodium hydroxide. In the second method the first solvent was aqueous fifth-normal barium hydroxide and the subsequent solvent was aqueous half-normal sodium hydroxide.

The first method was used as follows for the extraction of at least one sample of each liver. Forty grams of dry powder obtained from each of two amyloid-bearing livers and the same quantity of control material from one liver which had no deposits of amyloid were placed in separate flasks containing 750 cc. of aqueous half-normal sodium hydroxide. After extraction for eight hours at 10 C., the liquid was removed by centrifugation and replaced by an equal volume of the alkaline

TABLE 1.—Yields of Polysaccharide Obtained from Three Amyloid-Bearing Livers by Extraction with Barium Hydroxide and Sodium Hydroxide Singly or in Succession

Source	Tissue and Amount	Product	Percent Yield of Polysaccharide with Given Solvent		
			$\frac{N}{2}$ NaOH	$\frac{N}{5}$ Ba(OH) ₂	$\frac{N}{5}$ Ba(OH) ₂ Followed by $\frac{N}{2}$ NaOH
1	Liver, 40 Gm.	Barium salt	0.57	0.032	0.37
5	Liver, 40 Gm.	Barium salt	0.98	0.0	0.69
4	Liver, 40 Gm.	Barium salt	1.34	0.0	0.85

reagent. After twelve hours the second extract was recovered by centrifugation and the residue extracted for an additional twelve hours with 750 cc. of the alkaline reagent. After completion of the thirty-two hours of extraction, the three extracts were combined, neutralized with acetic acid and filtered. The filtrate was concentrated to a volume of 1,000 cc. by boiling. The concentrate was then made acid to litmus, an excess of barium carbonate was added, and boiling was continued until the volume was 400 cc. The precipitate and excess barium carbonate were then removed by centrifugation. The clear liquid thus obtained was reduced to a volume of 100 cc. by boiling and poured slowly while still hot into 900 cc. of cold glacial acetic acid. This solution was kept at 10 C. for twelve hours and the precipitate recovered by centrifugation. The precipitate was then dissolved in an excess of warm distilled water. This solution was filtered, concentrated to a small volume by boiling and poured into 9 volumes of glacial acetic acid. The biuret test on the precipitate was usually negative at this stage, but in all instances the precipitate was run through the steps of purification a third time and the biuret test applied again. The material was then collected on a sintered glass disk of fine porosity, washed with alcohol and ether, dried at 50 to 60 C. and weighed.

The second method of extraction was used on only one occasion. After the yields from 40 Gm. samples of three livers had been determined, a second set of

identical samples was extracted for seventy-two hours with successive volumes of aqueous fifth-normal barium hydroxide. The combined extracts were neutralized with acetic acid and concentrated to a volume of 400 cc. by boiling. The precipitate was removed by centrifugation and the supernatant fluid concentrated to a volume of 100 cc. This was poured while still hot into 900 cc. of glacial acetic acid. The precipitates were purified in the way used in the first method.

The residues of the hepatic tissue which had been extracted with aqueous fifth-normal barium hydroxide were then subjected to a second series of extractions with aqueous half-normal sodium hydroxide. The extracts were subsequently treated in the same way as extracts obtained by the first method. The comparative yields of purified material obtained by each method were determined.

The extraction of splenic tissue was done by the first method. There were six samples of dry pulverized splenic tissue. Only three spleens contained deposits of amyloid. Hence, three separate extractions were done, tissue from an amyloid-bearing spleen being carried through the procedure simultaneously with tissue from a normal spleen. About 20 Gm. of dry powder was available in each instance, so that the extraction with aqueous half-normal sodium hydroxide and subsequent steps were conducted with appropriate reductions in the volumes of reagents.

The dry powder obtained from infantile epiphysal cartilages was extracted in 5 Gm. lots and the product purified by the routine first method.

The preparation of the polysaccharide in the form of a free acid rather than as the barium salt obtained by methods which have been described required a few additional steps. Three preparations were made, two being obtained from hepatic tissue which gave the highest yields of the barium salt and one being obtained from cartilage. Forty grams of powdered liver and 5 Gm. of powdered cartilage were used in each instance. After recovery of the purified barium salt by the routine method, the salt was dissolved in a slight excess of warm water. Aqueous fifth-normal basic lead acetate was slowly added until precipitation was complete. The precipitate was separated by centrifugation, washed with water and decomposed in the cold with normal hydrochloric acid. The precipitate of lead chloride was removed by centrifugation and the supernatant fluid poured into 20 volumes of cold acetic acid. The precipitate which formed was collected on a porous glass filter, washed with alcohol and ether, and subsequently dried in a vacuum. In this way three samples of the acid with most of the base removed were obtained.

Methods of Chemical Analysis of Products.—The chemical analysis of each product was carried out as fully as permitted by the quantity of material. Whenever possible, each type of analysis was made simultaneously on samples of all products.

Ashing was done by the usual dry method. The ash was calculated in percentage as barium. Ten milligram samples of each product were used.

Nitrogen was determined by micro-Kjeldahl analysis on samples weighing 15 mg. Nitrogen was calculated in percentage on an ash-free basis.

Sulfur, recovered as sulfate, reducing substances in terms of dextrose and hexosamine in terms of glucosamine were determined by analysis of acid hydrolysates. The hydrolysis was conducted as follows: Fifteen milligram samples of each product were placed in separate pyrex tubes. Five cubic centimeters of four times normal hydrochloric acid and 0.1 cc. of half-normal barium chloride were added to each tube. The tubes were sealed in a flame and immersed in boiling water for seven hours. Then the tubes were cooled, the sealed tips broken and the hydrolysates filtered into graduated centrifuge tubes. The precipitates collected on the filter papers were preserved for ashing. The filtrates were diluted to 10 cc. One cubic centimeter of each filtrate was pipetted into a graduated centrifuge tube

and neutralized with aqueous half-normal sodium hydroxide. Each neutralized sample was diluted to 10 cc. One cubic centimeter was used for determination of reducing substances by the micromethod of Folin.¹ An identical volume was used for the determination of hexosamine by the method of Elson and Morgan² with modifications suggested by Palmer and co-workers.³ Total reducing substances were calculated in terms of dextrose, and hexosamine was calculated in terms of glucosamine. All percentages were referred to the ash-free basis.

The precipitates which formed during hydrolysis were collected on filter papers, dried and ashed. The solubility of the ash in concentrated hydrochloric acid before and after fusion with sodium carbonate was determined. The result led to calculation of the ash as barium sulfate, and sulfur, recovered as sulfate, was calculated in percentage on an ash-free basis.

Acetyl determinations were done on 20 mg. samples of each product. These were hydrolyzed with paratoluene-sulfonic acid. The acetic acid which was formed was distilled from the hydrolysates in a current of steam and the distillate titrated with aqueous fiftieth-normal sodium hydroxide. Calculations were made on an ash-free basis.

Determinations of uronic acid anhydride were made on samples weighing 200 mg. by the method of Dickson, Otterson and Link.⁴

The acid equivalent was determined on 20 mg. samples of the free acid, which was dissolved in water and titrated with aqueous fiftieth-normal sodium hydroxide to the end point of methyl red.

RESULTS

Morphologic Characteristics of Amyloid Deposits in the Tissues.—

The gross appearance of six livers and three spleens was characteristic of that commonly noted in tissues when amyloid deposits are present in large amounts. The translucent firm amyloid deposits in two livers and one spleen were salmon pink. This color was due to congo red, which had been injected intravenously for diagnostic purposes prior to death of the patients. Three livers and three spleens were grossly normal.

Microscopic study of fresh frozen sections disclosed amyloid deposits only in those tissues in which the deposits were detected by gross examination. Hence three livers and three spleens, obtained from patients with chronic pulmonary tuberculosis, contained no microscopically recognizable amyloid deposits and were regarded as suitable for control analyses.

The quantity of amyloid in each organ as estimated by a microscopic study of the tissues is recorded in table 2. The amount of amyloid varied among the different organs and to a slight extent in different parts of the same organ. It was estimated, by a microscopic survey of several blocks of tissue from each organ, that the minimum quantity of

1. Folin, O.: *New England J. Med.* **206**:727, 1932.

2. Elson, L. A., and Morgan, W. T. J.: *Biochem. J.* **27**:1824, 1933.

3. Palmer, J. W.; Smyth, E. M., and Meyer, K.: *J. Biol. Chem.* **119**:491, 1937.

4. Dickson, A. D.; Otterson, H., and Link, K. P.: *J. Am. Chem. Soc.* **52**:775, 1930.

amyloid in any liver was about 20 per cent and that the maximum quantity was not less than 90 per cent of the weight of the organ. About one half of the total bulk of each spleen was composed of amyloid.

The distribution of the deposits of amyloid followed a uniform pattern in each liver, and any microscopic differences were due to the variable degree of replacement of normal lobular structure by the sub-sinusoidal accumulations.

TABLE 2.—*Yields and Chemical Characteristics of the Polysaccharides Isolated from Amyloid-Bearing Tissues and Cartilage*

Experiment	Source	Tissue and Amount	Amyloid, % *	Product	Yield, %	Ash, %	Nitrogen, % †	Sulfur, % †	Reducing Substances, % †	Hexosamine, % †	Uronic Acid Anhydride, % †	Acetyl, % †	Acid Equivalent †
1	1	Liver, 40 Gm.	40	Barium salt	0.57	24.16	4.10	4.62	55.1	32.2
2	2	Liver, 40 Gm.	90	Barium salt	1.71	19.88	3.87	5.01	58.6	34.1	35.0	6.4	...
3	3	Liver, 40 Gm.	20	Barium salt	0.22	18.63	3.14	5.29	63.5	30.1
4	4	Liver, 40 Gm.	70	Barium salt	1.34	22.26	3.92	5.41	54.6	32.2	36.8	10.4	...
5	5	Liver, 40 Gm.	50	Barium salt	0.98	20.76	3.42	5.93	56.9	34.8
6	6	Liver, 40 Gm.	60	Barium salt	1.33	18.79	2.96	5.26	69.2	39.6	40.6	8.0	...
7	2	Spleen, 16 Gm.	40	Barium salt	0.34	19.41	3.18	4.82	64.1	36.5
8	4	Spleen, 20 Gm.	40	Barium salt	0.31	18.04	3.27	4.49	66.6	35.0
9	7	Spleen, 20 Gm.	50	Barium salt	0.25	20.04	3.20	4.71	60.2	38.1
10	13	Cartilage, 5 Gm.	0	Barium salt	21.42	18.23	3.28	4.08	61.4	37.3	42.1	7.6	...
11	2	Liver, 40 Gm.	90	Free acid	1.18	0.46	3.19	4.96	64.6	36.0	38.3	11.1	386
12	4	Liver, 40 Gm.	70	Free acid	0.96	0.21	3.06	5.14	68.3	38.6	370
13	13	Cartilage, 5 Gm.	0	Free acid	16.70	0.11	3.01	3.92	67.0	36.9	8.9	410
14	7, 8, 9	Control livers, 40 Gm.	0	Barium salt	0.0								
15	10, 11, 12	Control spleens, 20 Gm.	0	Barium salt	Trace								

* Each value represents a microscopic estimate of the quantity of amyloid.

† Each value was calculated on an ash-free basis.

The amyloid deposits in two spleens were essentially restricted to sites formerly occupied by lymph follicles. In the third spleen, deposits not only were present in the follicular and perifollicular regions but also were diffusely distributed between the vascular sinusoids of the interfollicular splenic structure.

Amyloid deposits were present in the walls of small arterioles in one liver and one spleen.

Wherever the amyloid deposits were found, they possessed equal classic affinities for certain dyes. The brown color acquired in the presence of compound solution of iodine was prominent in all instances. All deposits were stained red with crystal violet and had a strong affinity

for congo red. There were no birefringent materials in the deposits, and fibrillar structure was not distinguishable.

Notes on Extraction and Purification of the Polysaccharide.—During extraction of splenic or hepatic tissue with aqueous half-normal sodium hydroxide, the solvent acquired color and high viscosity. The viscosity was no greater with amyloid-bearing tissues than with normal tissues. The intensity of the yellowish brown color acquired by the solvent was inversely proportional to the amount of amyloid in the tissue. This color was obscured by a red color in the 3 instances in which the amyloid deposits had absorbed and retained congo red during life.

The quantity of the tissue residue after completion of the extraction was inversely proportional to the amount of amyloid initially present in the sample. All color attributable to congo red disappeared from the residue during extraction, and at the same time the iodine and crystal violet reactions of the tissue became negative.

During the seventy-two hours of extraction with aqueous fifth-normal barium hydroxide little material was dissolved from either the normal or the amyloid-bearing hepatic tissue. This solvent did not extract the red color from hepatic tissue which was vitally stained with congo red. The subsequent extraction of this tissue, however, with aqueous half-normal sodium hydroxide removed the dye from the residue and otherwise yielded solutions similar to those obtained by initial extraction with this solvent.

Neutralization of the extracts did not lead to formation of a precipitate. The extracts acquired turbidity during concentration by boiling, but a significant degree of precipitation did not occur until barium carbonate was added. The precipitate which formed at this time was usually more voluminous when the solution was an extract of amyloid-bearing tissues. When the extracts had a red color attributable to congo red, this color disappeared from the solution on addition of barium carbonate and was acquired by the precipitate.

The precipitation of the proteins with barium reduced the viscosity of solutions. The residual viscosity was not a suitable index for determining whether the solution was an extract of normal or of amyloid-bearing tissue. The index by which this determination could be made became apparent when the clear concentrated deproteinized extracts were poured into acetic acid. In this medium a flocculent white precipitate formed within a few minutes in a quantity which was directly proportional to the quantity of amyloid in the tissue from which the extract was obtained. Only a trace of precipitate formed when normal splenic or hepatic tissues were sources of material for extraction. This quantity, though proportionately larger when splenic tissue was the source of normal material, was so small that it was reduced almost to zero on subsequent purification.

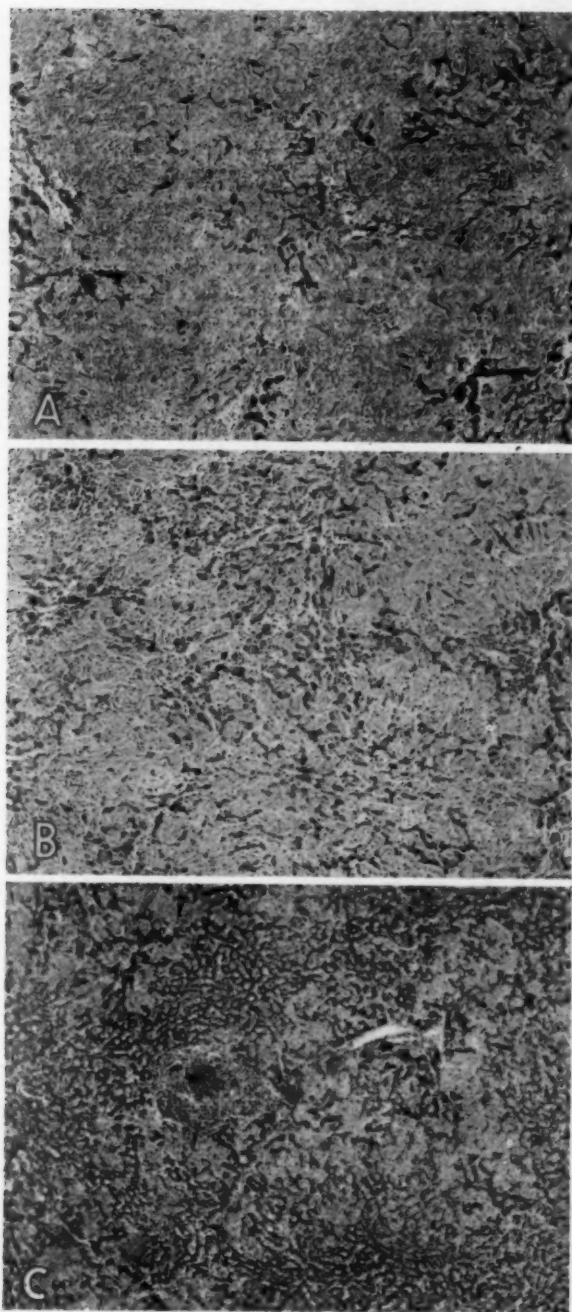


Figure 1

(See legend on opposite page)

The purified products were dry white powders. They were slightly hygroscopic and dissolved easily in water to form viscous transparent opalescent solutions. Biuret tests in low dilutions were negative.

The conversion of the barium salt to the free acid was not complete in any instance. Owing to the high solubility and instability of the free acid, this conversion had to be carried out quickly in the cold. As a consequence, no product was ash free, and manipulative losses were considerable.

The products obtained from cartilage as the barium salt or the free acid were physically similar to those isolated from amyloid-bearing tissues. The impression was gained that aqueous solutions of these products had a lower viscosity than comparable solutions of materials obtained from amyloid-bearing tissue.

Relations Between Yields of Polysaccharide and Quantities of Amyloid.—The relative merits of aqueous fifth-normal barium hydroxide and half-normal sodium hydroxide as solvents for extraction of the polysaccharide are shown in table 1. The yields with barium hydroxide were negligible. Prolonged extraction with this reagent reduced the expected yields when the tissue was subsequently extracted with aqueous half-normal sodium hydroxide.

The yields when aqueous half-normal sodium hydroxide was the only solvent are recorded in table 2. A comparison of these figures with the relevant values in table 2 shows that there was a direct dependence of each yield on the amount of amyloid as estimated microscopically. This dependence never assumed the proportions of a fixed ratio. In general, the yields were disproportionately low when the tissue contained small amounts of amyloid and when splenic tissue was the source of material for study.

Among the several organs, source 2, which is illustrated in figure 1 *A*, contained the largest quantity of amyloid. It was estimated microscopically that about 90 per cent of the bulk of this liver was amyloid. The yields of 1.71 per cent of the tissue as polysaccharide in

EXPLANATION OF FIGURE 1

A, photomicrograph of liver, source 2, showing about 90 per cent replacement of hepatic parenchyma by deposits of amyloid. The yield of polysaccharide as a barium salt was 1.71 per cent.

B, photomicrograph of liver, source 4, showing about 70 per cent replacement of hepatic parenchyma by deposits of amyloid. The yield of polysaccharide as a barium salt was 1.34 per cent.

C, photomicrograph of liver, source 3, showing about 20 per cent replacement of hepatic parenchyma by deposits of amyloid. The yield of polysaccharide as a barium salt was 0.22 per cent.

the form of the barium salt and 1.18 per cent as a reasonably pure acid were the maximum yields among the several tissues.

Source 4, which is illustrated in figure 1 *B*, likewise contained a large amount of amyloid. Though the amount varied considerably from place to place in this liver, it was estimated that no less than 70 per cent of the tissue was composed of amyloid. The yields of 1.34 per cent of the tissue as a polysaccharide in the form of a barium salt and 0.96 per cent in the form of a free acid were in good agreement with those obtained from source 2.

Source 3, which is illustrated in figure 1 *C*, contained a quantity of amyloid which was estimated to be about 20 per cent of the tissue. This percentage was the lowest among the several organs subjected to analysis, and the tissue gave the lowest yield of the barium salt of the polysaccharide, namely, 0.22 per cent.

The three spleens contained about the same amounts of amyloid, and the percentage of each tissue recovered as the barium salt of the polysaccharide was nearly the same. Sources 4 and 7 are illustrated, respectively, in figure 2 *B* and *A*.

In view of these semiquantitative correlations it seems that the minimum quantity of pure deposits of amyloid recovered as a polysaccharide was not far removed from the maximum quantity. The maximum quantity may be given on the ash-free basis as 1.5 per cent. This value is to be compared with yields of 15 to 17 per cent of a similar polysaccharide from various samples of human epiphyseal cartilage that I have studied.

Chemical Characteristics of the Polysaccharide Isolated from Amyloid-Bearing Tissues.—The chemical characteristics of the products from each source are recorded in table 2. It is not necessary to discuss the meaning of the variations among the different samples. It is probable that incomplete conversion of the acid or the sodium salt to the barium salt was responsible for some differences among values. If the hydrolysis of samples with hydrochloric acid had been less prolonged, the values for total reducing substances would have been higher, because glycuronic acid progressively decomposes during hydrolysis. The prolonged period was chosen because it was more suitable for obtaining high values for hexosamine. The sum of the hexosamine and uronic acid anhydride percentages may be taken as a closer approximation to the total percentage of reducing substances. Some nitrogen and sulfur values were high and all samples doubtless contained more or less impurities. Nevertheless, the analyses were in fair agreement with those of interstitial sulfate-bearing polysaccharides, among which chondroitin-sulfuric acid is an example.

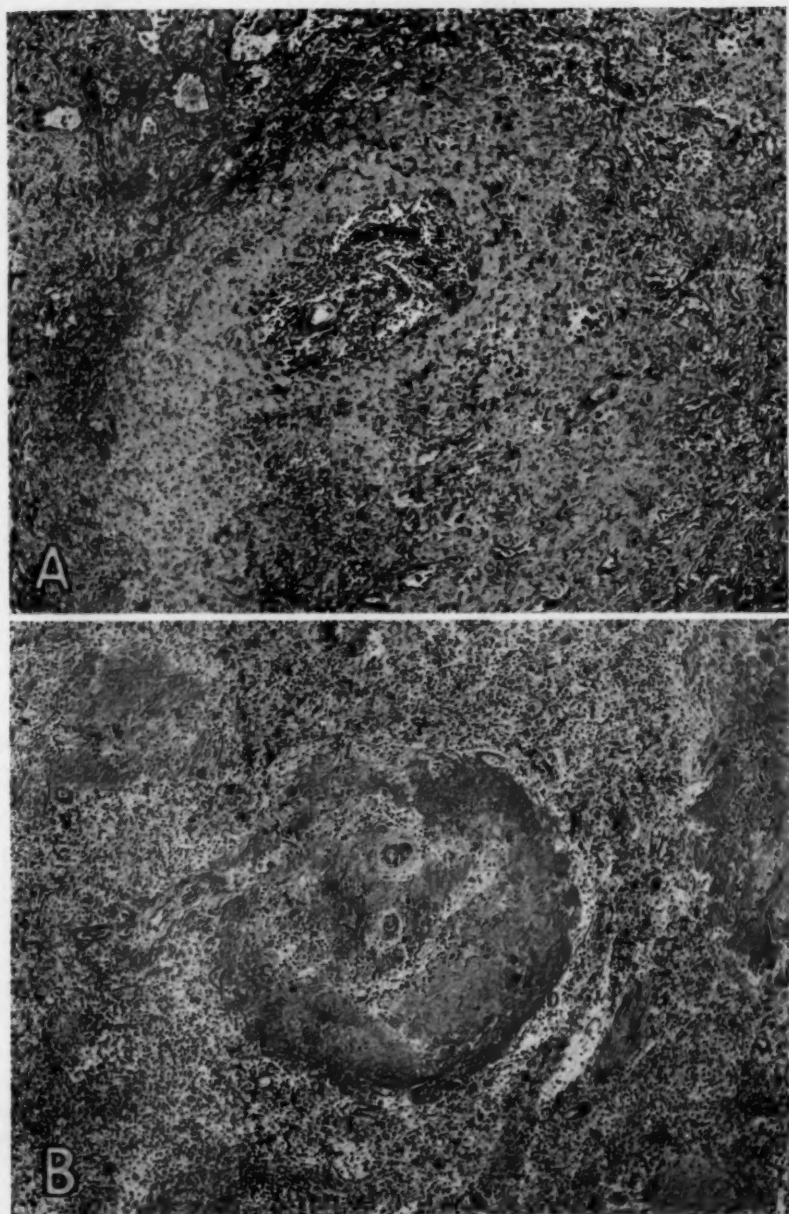


Fig. 2.—*A*, photomicrograph of spleen, source 7, showing diffuse perifollicular and intercapillary amyloidosis with replacement of an estimated 50 per cent of the bulk of the tissue with amyloid deposits. The yield of polysaccharide as a barium salt was 0.25 per cent.

B, photomicrograph of spleen, source 4, showing follicular amyloidosis with estimated replacement of 40 per cent of the bulk of the tissue by amyloid. The yield of polysaccharide in the form of a barium salt was 0.31 per cent.

COMMENT

There are two fundamental aspects to the study of amyloid disease. One is genetic and is concerned with the factors which govern the formation and deposition of the amyloid matrix. The second aspect is in a sense environmental and is concerned with interactions in time between the initial deposits and the components of the medium which bathes and permeates them.

The factors which govern the formation and deposition of the amyloid matrix are unknown. Whatever they may be, it may be assumed that they are components of an abnormal system, and, there are reasons for believing that this system is concerned with vital interactions between antigens and immune bodies. In accord with this belief, I choose to regard amyloid deposits as antigen-antibody precipitates and to imply that the deposits which occur in diverse diseases are not identical, that they are compounds of circulating soluble materials precipitated in the

TABLE 3.—*Elementary Analyses of Amyloid Protein Fraction A*

Source of Protein Fraction A	Tissue	C	H	N	S	S as SO ₄	P
2.....	Liver	51.48	6.84	16.41	0.90	0.28	0.01
6.....	Liver	51.52	6.87	16.48	0.64	0.21	0.00

tissues by specific immunologic mechanisms and that once precipitated they are susceptible to modification by the environmental medium.

The proof of this idea would be simplified if the reactions between the hypothetic soluble materials were sufficiently reversible to permit recovery of these materials in unmodified form. Though reversibility in an immunologic sense could hardly be expected, a study was undertaken with the intention of dissolving amyloid and recovering as many complex degradation products as possible.⁵ This study showed that deposits of amyloid in organs obtained from patients with tuberculosis had a sharp solubility end point at p_H 11. Hence, by extraction of fresh frozen sections of hepatic tissue containing a high percentage of amyloid successively at p_H 7, p_H 10 and p_H 11, a final extract was obtained which contained only those materials soluble in the range p_H 10 to 11. In the ideal case these materials were derived almost exclusively from amyloid. Fractionation of the extractives obtained at p_H 11 resulted in recovery of a principal protein fraction in reasonably pure form. This fraction, isolated in large amounts from two favorable sources, has been subjected to elementary analysis. The analyses are given in table 3.

5. Hass, G., and Schulz, R. Z.: Arch. Path. **30**:240, 1940.

The behavior of this fraction indicated that the protein alone, unless it was unduly modified by the method of isolation, was not responsible for the solubility properties of the natural amyloid matrix. It seemed reasonable, therefore, to search for some material which by combination with the protein would yield a product with properties of the matrix. Inasmuch as the segregation of components of protein-protein complexes is a questionable undertaking, especially in dealing with tissue structure, an effort was made to isolate a nonprotein component from extracts of amyloid-bearing tissues.

The direction of this effort was conditioned by knowledge of the solubility end point of amyloid and by conflicting reports in the literature concerning the presence of chondroitin-sulfuric acid in amyloid deposits. In 1894 Oddi studied two amyloid-bearing livers.⁶ Certain fractions gave qualitative reactions for reducing substances and sulfate. The conclusion was reached that the fractions contained chondroitin-sulfuric acid. In 1898 Krakow undertook a more extensive study of the problem.⁷ Eight livers, one spleen and one kidney from patients who had died of unrecorded diseases were analyzed. The products which were isolated were characterized by qualitative reactions for reducing substances and sulfate. There were no quantitative morphologic or chemical data. The conclusion was reached that chondroitin-sulfuric acid was present in amyloid-bearing organs. In 1908 Hanssen isolated granules of amyloid from splenic tissue by mechanical methods. These granules were pooled and analyzed for their sulfate content. In view of the small amounts of sulfate which were recovered, Hanssen concluded that no chondroitin-sulfuric acid was present in deposits of amyloid.⁸ His study of amyloid-bearing organs, however, led to a possible contradiction of the conclusion. Splenic tissue with deposits of amyloid averaged 0.09 per cent more sulfate than normal splenic tissue. Similar differences in sulfate values were found during a study of amyloid-bearing and normal hepatic tissue. Hence, his conclusion was qualified by the statement that the differences among sulfate values, if due to chondroitin-sulphuric acid, would be in accord with a content of 2 per cent of the polysaccharide. In 1922 Eppinger analyzed an amyloid tumor in the liver of a young patient who had no disease which could be held responsible for the amyloidosis.⁹ The tumor contained no phosphorus, sulfur or qualitatively detectable carbohydrate. The conclusion was reached that no chondroitin-sulfuric acid was present in the amyloid tumor.

6. Oddi, R.: *Arch. f. exper. Path. u. Pharmacol.* **33**:376, 1894.

7. Krakow, N. P.: *Arch. f. exper. Path. u. Pharmacol.* **40**:195, 1898.

8. Hanssen, O.: *Biochem. Ztschr.* **13**:185, 1908.

9. Eppinger, H.: *Biochem. Ztschr.* **127**:107, 1922.

The present studies show that a compound similar to those belonging to the class of interstitial sulfate-bearing polysaccharides can be recovered from amyloid-bearing hepatic and splenic tissue of patients with tuberculosis. There was a direct proportion between the amount of amyloid in the tissues and the yield of the polysaccharide. This proportion was not a fixed ratio, and traces of the polysaccharide were isolated even from control normal tissues of patients who had died with chronic pulmonary tuberculosis. Nevertheless, the isolation of large quantities of the polysaccharide from organs containing a large amount of amyloid was presumptive evidence that the polysaccharide was principally confined to the amyloid matrix. This evidence was strengthened by proof that the polysaccharide was recovered only under those conditions of extraction which dissolved the amyloid matrix.

The yields of the polysaccharide were such that 1.5 per cent may be given as the maximum quantity of the product as an acid in amyloid deposits. The minimum quantity was about 0.5 per cent. Neither percentage is an absolute value. Larger yields should be obtainable with less drastic methods, and it is probable that a fairly constant yield could be obtained if every variable inherent in the source material and methods could be controlled. One variable which definitely reduced the yield under controlled conditions was a preliminary treatment of the tissue with aqueous fifth-normal barium hydroxide prior to extraction with aqueous half-normal sodium hydroxide. There was no apparent cause for this undesirable action of barium. A second variable which might have reduced the yield in the presence of a less concentrated solution of sodium hydroxide was vital staining with congo red, for it has been shown that the solubility of amyloid is reduced when the matrix is stained with this dye.⁵ A third variable was the amount of amyloid in the tissue. This variable had an apparent effect, because the smaller the quantity of hepatic amyloid the lower was the apparent content of polysaccharide in the amyloid. It is not probable that this result was due to any essential difference in the composition of the amyloid. On the contrary, it was probably due to larger fractional losses of the polysaccharide during purification of small amounts of the initial crude products. These losses, likewise, would easily account for the low percental yields of the polysaccharide from splenic amyloid. Hence, it seems that the polysaccharide fraction may be regarded as constant in quantity in the type of amyloid with which these studies were concerned.

Chemical analysis of the products from the different sources showed that they were similar to one another and to chondroitin-sulfuric acid isolated from normal cartilage. Similarity was all that was demonstrated, because identity among these complex polymeric compounds is as difficult to prove as identity among proteins. It is possible that additional

chemical studies may reveal that the amyloid polysaccharide possesses distinctive qualities, but biologic studies might serve as a more suitable method for discovering any singular properties. For instance, crude heparin is not easily distinguished by ordinary chemical methods from the interstitial sulfate-bearing polysaccharides, but it has biologic properties which they do not possess. For this reason and because of the elective distribution of amyloid beneath the endothelium of capillaries, a study of the anticoagulant properties of the amyloid polysaccharide should be undertaken. Other studies, too numerous to consider in a speculative way, are indicated with due regard for the possible role of the principal protein fraction, previously isolated.

SUMMARY

A polysaccharide was isolated by semiquantitative methods from amyloid-bearing spleens and livers obtained post mortem from patients with chronic pulmonary tuberculosis.

Control hepatic and splenic tissues from similar sources but without microscopic deposits of amyloid did not contain significant amounts of the polysaccharide.

The compound was recovered only under conditions which led to solution of the amyloid matrix. The amount which was isolated was directly proportional, within limits of error, to the estimated quantity of amyloid. The yields indicated that no more than 1.5 per cent of the amyloid matrix in any instance was composed of the polysaccharide.

In properties and principal degradation products the preparations were quantitatively and qualitatively similar not only to one another but also to samples of chondroitin-sulfuric acid obtained from infantile cartilage.

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USE OF THE SUPRAVITAL STAINING TECHNIC IN THE STUDY OF TUMORS OF THE LYMPHOSARCOMA GROUP

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The usefulness of the supravital staining technic in the study of blood and connective tissue cells in both health and disease has been demonstrated by many workers.¹ This technic as described by Sabin,² Forkner¹⁸ and others has a number of advantages over the ordinary methods of study of fixed cells. It demonstrates the mitochondria, granules and vacuoles which are usually unrecognizable or inconspicuous in ordinary preparations; it permits direct observation of such activities as locomotion and phagocytosis of certain cells; it differentiates with a greater degree of precision the monocytes from the clasmatoocytes (histiocytes), the stimulated cells from the resting cells and the old cells from the young.

Although this technic has been used with success for the study of blood cells and the cells of connective tissue, no attempt has been made, except by McJunkin,¹⁸ to apply it to the study of tumors of the blood-forming organs, in which the differentiation between certain types of tumor cells is often difficult. It may be of interest, therefore, to report the observations made on three tumors of the lymphosarcoma group which were studied by this technic in combination with the routine histologic method.

From the Department of Pathology, Peiping Union Medical College.

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2. Sabin, F. R.: *Bull. Johns Hopkins Hosp.* **34**:277, 1923.

REPORT OF CASES

CASE 1.³—A 28 year old Chinese woman came to the outpatient department of the Peiping Union Medical College Hospital on June 27, 1938, complaining of a tumor present in the right side of the neck for two years. It was slow growing at first, but during the last five months it had grown rapidly. At the time of examination the tumor measured about 10 by 7 cm., was located in the upper cervical region on the right side and seemed to be composed of several tender firm masses fixed to the underlying tissue. The tumor was treated as tuberculous lymph nodes for more than two months, during which time it was incised but yielded no pus. Instead of healing, the wound became larger, so that by September 12 it was 7 cm. in diameter and 2 cm. deep. Then a biopsy specimen was obtained for histologic examination, and the diagnosis of monocytic sarcoma was made. A course of treatment with roentgen rays was given, the total dose being 1,672 roentgens (r). Following the treatment, the induration disappeared, the pain was greatly decreased and the ulcer was reduced to 5 cm. in diameter. Eventually, after application of a skin graft, the wound healed completely. When the patient was seen on Feb. 21, 1939, no evidence of recurrence was found.

The blood picture before the institution of roentgen therapy was as follows: red blood cells, 4,180,000; white blood cells, 10,800 per cubic millimeter of blood; hemoglobin, 13.7 Gm. per hundred cubic centimeters of blood. Differential counts by the supravital technic showed neutrophils 69 per cent, lymphocytes 12 per cent, monocytes 12 per cent, metamyelocytes 4 per cent, eosinophils 1 per cent and unclassified cells 2 per cent.

Examination of the Tumor Tissue.—The biopsy specimen consisted of a small bit of grayish white soft tissue about 8 mm. in diameter. It presented two parts, the superficial granulation tissue and the deeper tumor tissue. The latter consisted of numerous large cells with abundant cytoplasm and rounded, oval, indented or kidney-shaped vesicular nuclei. After hematoxylin-eosin staining, the cytoplasm was pale pink without distinct cell borders (fig. 1 B). Some cells seemed to join with their neighboring cells by cytoplasmic processes. Occasionally one might observe, especially in the cells with indented nuclei, slight condensation of the cytoplasm opposite the indentation of the nucleus. Many mitotic figures were present.

For supravital staining of the tumor cells a minute bit of the tumor tissue was removed from the biopsy specimen while it was still very fresh. The tissue was then placed, together with a drop of physiologic solution of sodium chloride, on a clean slide previously coated with neutral red, and a cover slide was placed on top of it. By pressing gently on the cover slide, the cells from the tissue were spread out, and after standing a few minutes at room temperature, they had absorbed the stain and were ready for examination.

In the tissue so prepared (fig. 1 A) many cells containing rosettes of neutral red bodies and resembling the monocytes described by Sabin, Doan and Cunningham²¹ were found. Many of the rosettes were large and conspicuous, but some of them were small and inconspicuous, consisting of only a few fine neutral red bodies. There also were cells which did not show any neutral red bodies, and such cells usually had rounded nuclei, whereas those with rosettes had indented or

3. This case has been reported by one of us (C. H. H.) in the Chinese Medical Journal (1940, supp. 3, p. 26).

kidney-shaped nuclei. The cells containing no rosettes were considered as primitive undifferentiated cells; those with poorly formed rosettes, as immature monocytes. The oxidase reaction of all the cells was negative in frozen sections.

The diagnosis was monocytic sarcoma.

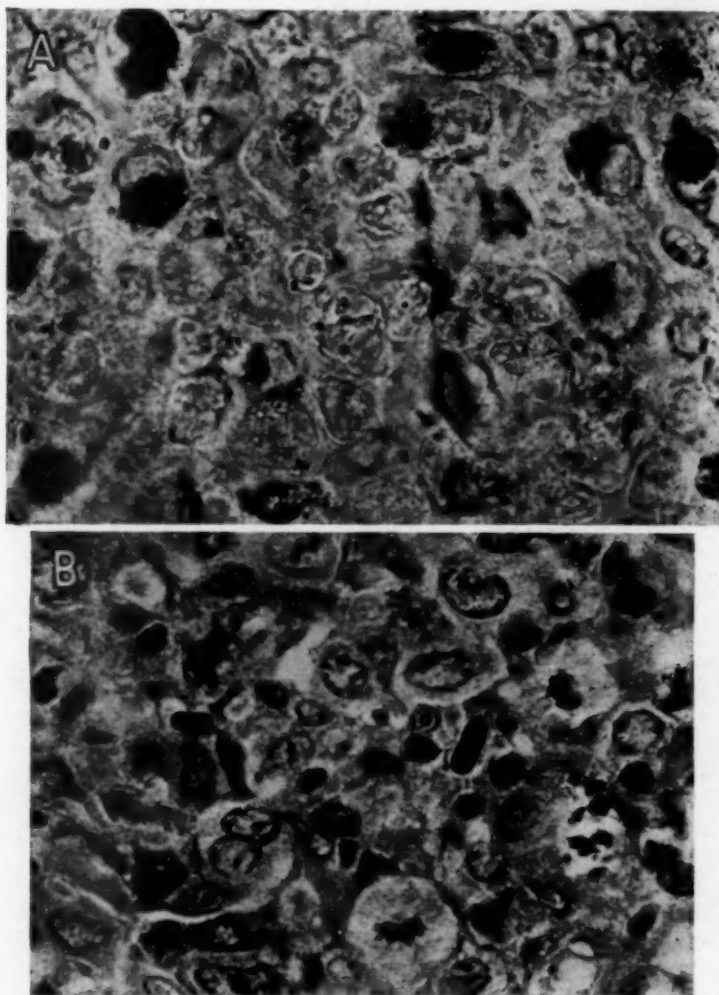


Fig. 1 (case 1).—*A*, tumor tissue showing monocytes with neutral red rosettes. The rosettes of the younger monocytes are inconspicuous in the photograph. Supravital staining; $\times 1,000$. *B*, tumor tissue showing a mixture of reticulum cells and monocytes with kidney-shaped nuclei. Hematoxylin and eosin; $\times 1,000$.

From the histologic appearance of the microscopic sections one would classify the tumor as reticulum cell sarcoma in which some of the reticulum cells had become differentiated into monocytes with indented or kidney-shaped nuclei. This differentiation was much more strikingly

brought out in the supravital stained preparations, in which the intermediate forms with imperfectly formed rosettes could be recognized easily. The combined use of the supravital staining technic and the routine methods of histologic study is therefore of great value in making easier the recognition of tumors of this type.

CASE 2.—A 9 year old Chinese girl was seen on July 1, 1940 because of rapidly growing masses present on both sides of the neck for seven months. Examination revealed a firm, fixed and tender rubbery mass, measuring 12 by 8 cm., on the right side of the neck, a movable mass, measuring 8 by 8 cm., on the left side of the neck and a few firm, freely movable nodules in the right midcervical region and in the left axilla. Other findings were unimportant. Material for biopsy was obtained by needle puncture, and a diagnosis of "degenerated tumor tissue, probably lymphosarcoma," was made.

On July 3 the patient was admitted to the hospital and was treated with high voltage roentgen radiation. The response, however, was not very prompt, and the treatment was discontinued after 800 r had been delivered to each side of the neck. The patient was discharged on July 15.

On July 31 she was readmitted, and on August 3 a small nodule near the tumor on the left side of the neck and a small piece of the main tumor were removed. Additional high voltage radiation was administered, but the tumor again did not show much regression; so the treatment was discontinued on August 26, after a total of 2,380 r had been given.

On August 27 a nodule was removed from the right axilla, to which no radiation had been applied. After the discontinuance of the treatment, the tumor masses in the neck and the axillas rapidly increased in size. Swallowing and breathing became difficult. A tumor was noticed in the right lower quadrant of the abdomen on August 30. On September 28 the patient had a high fever, and signs of pneumonia developed in the lower lobe of the right lung. By the next day, all the tumors had decreased in size, and the abdominal mass no longer could be felt. The patient died on October 12.

The blood findings were: red blood cells, 3,550,000 to 3,970,000; white blood cells, 6,000 to 9,400 per cubic millimeter of blood; hemoglobin, 9 to 11.5 Gm. per hundred cubic centimeters. The differential counts showed neutrophils 78 to 84 per cent, lymphocytes 10 to 15 per cent, eosinophils 4 to 5 per cent, basophils 0 to 1 per cent and monocytes 1 to 2 per cent.

At autopsy, tumor masses and nodules were found in the lymph nodes of the neck, the axillas, the mediastinum, the retroperitoneum and the inguinal regions; tumors also were present in the bone marrow, the ovaries and the uterus.

Examination of the Tumor Tissue.—The first specimen, obtained by needle on July 1, showed a diffuse growth consisting of necrotic and partially degenerated round cells. No healthy tissue was present.

The second specimen, obtained on August 3, consisted of a small piece of tissue from the main tumor, measuring about 1 cm. in its greatest diameter, and two tiny nodules, each measuring about 0.5 cm. in diameter. Microscopically, the tissue from the main tumor showed a diffuse growth of rounded cells (fig. 2A). The cytoplasm of these cells was scanty, the nuclei were rounded and rich in chromatin, and the nuclear membrane was prominent. They were morphologically indistinguishable from lymphocytes. Most of them were medium sized, but a few were large and a few small. Mitoses were frequently found. There were also a few reticulum cells and many degenerated cells. The tissue from the nodules showed a

small lymph node, the structure of which was largely destroyed by a diffuse infiltration of reticulum cells, which had no cell boundaries but were either fused together or interconnected by cytoplasmic processes (fig. 2 *B*). The nuclei were

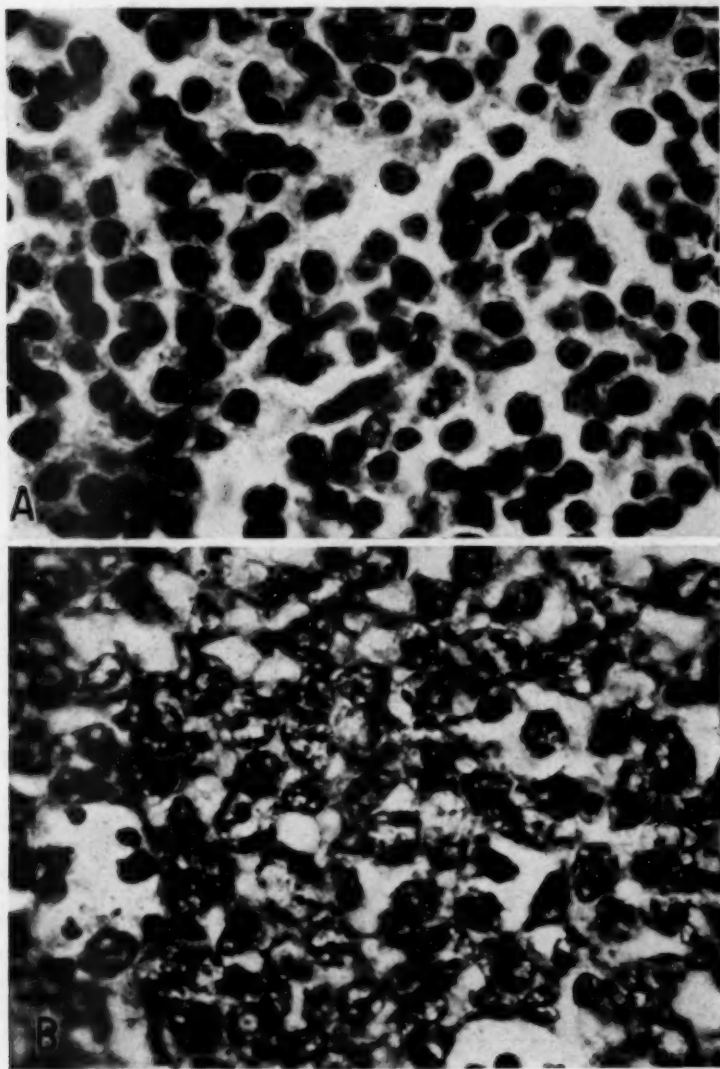


Fig. 2 (case 2).—*A*, tissue from the main tumor showing medium-sized dark-staining lymphoid cells with rounded nuclei and scanty cytoplasm. Hematoxylin and eosin; $\times 1,000$. *B*, tumor tissue from the small lymph node showing a diffuse growth of the reticulum cells. Hematoxylin and eosin; $\times 1,000$.

large, rounded or oval, vesicular, often poorly stained and crowded together or overlying each other. Mitoses were fairly frequent. A few degenerated cells were found among the infiltrating cells.

The third specimen, a lymph node 3.5 cm. in diameter removed on August 27, was studied with the supravital staining technic as well as by the ordinary methods. The cells were obtained by gently scraping with a cataract knife the freshly cut

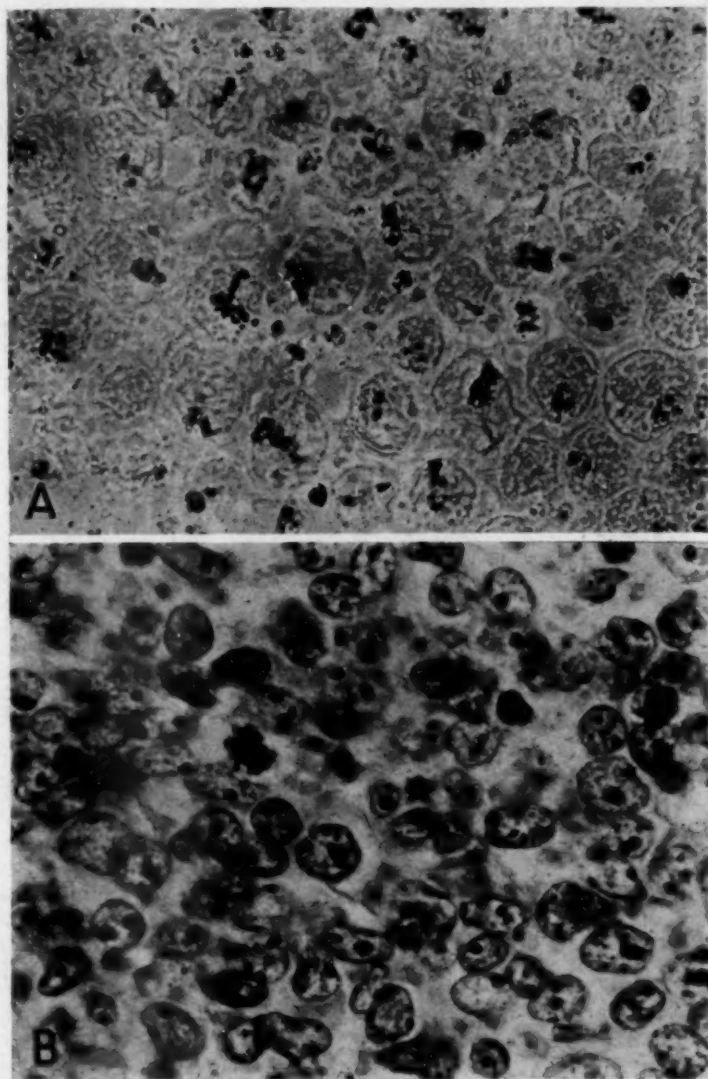


Fig. 3 (case 2).—*A*, tumor cells from the node removed from the right axilla. There are conspicuous collections of neutral red bodies in most of the cells opposite the notches in the nuclei. Supravital staining; $\times 1,000$. *B*, tumor tissue from the peripheral portion of the node removed from the right axilla, showing chiefly large reticulum cells with vesicular nuclei and monocytes with indented nuclei. Hematoxylin and eosin; $\times 1,000$.

surface of the tumor and were then transferred to a stained slide with a small drop of saline solution. In the film so prepared there were many medium-sized and large rounded cells with slightly indented rounded nuclei (fig. 3 *A*). Cytoplasm was present only in a small or moderate amount; a group of neutral red bodies was found in the cytoplasm in the region of the indentation of each nucleus, suggesting rosette formation. A small number of mitochondria were observed around the nuclear border or at the periphery of the neutral red collections. For the lack of a better term they were designated as monocytoid lymphocytes. Among these monocytoid lymphocytes there also were numerous typical monocytes and undifferentiated primitive cells or reticulum cells.

Microscopic examination of sections of the same tumor showed that the normal structure of the lymph node was entirely obliterated by a diffuse growth of tumor cells, which were of two main types. Those of the first type were located chiefly

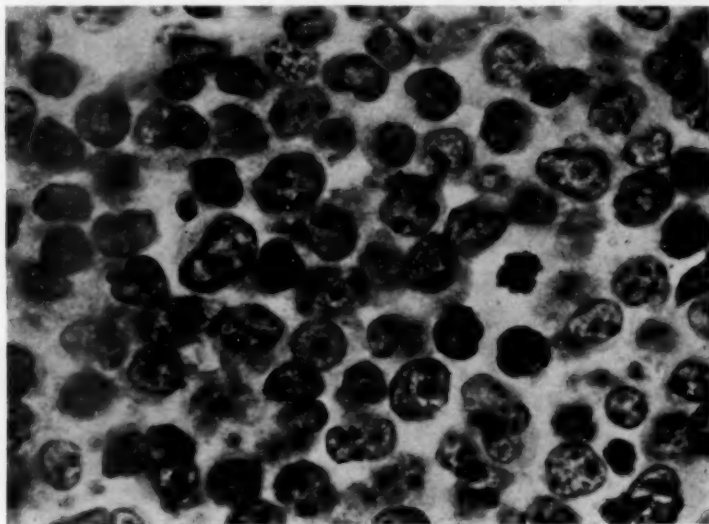


Fig. 4 (case 2).—Tumor tissue from the central portion of the node. The cells are like lymphoid cells; their nuclei are more rounded and more hyperchromatic, but nuclear indentation is still noticeable in many of them. Hematoxylin and eosin; $\times 1,000$.

near the periphery of the lymph node. They were large cells with vesicular rounded, oval, elongated or well indented nuclei and comparatively abundant pale cytoplasm, which in some cells had no distinct boundary. These cells were believed to represent the reticulum cells and the monocytes (fig. 3 *B*). As one passed from the periphery of the lymph node to its central portion, one noticed a gradual transition from the cells of the first type to those of the second type (fig. 4). The nuclei of these cells were large, moderately hyperchromatic and rounded, with only slight or moderate indentation. Their cytoplasm was not abundant but was moderately deeply stained. The cell boundary was distinct. These cells resembled large and medium-sized lymphocytes and apparently corresponded to the monocytoid lymphocytes observed in the supravital stained films (fig. 3 *A*).

The material obtained at autopsy was partly decomposed, so that the histologic study of the tumors was not satisfactory. As far as could be made out, both the cells from the main tumor and those from the secondary growths were chiefly monocytoïd lymphocytes. In one lymph node removed from the right axilla (not irradiated) a number of multiple-nucleated tumor cells were found.

The oxidase reaction of the tumor cells was negative.

The diagnosis was lymphosarcoma (monocytoïd lymphocytic type).

There were several types of cells in this tumor: reticulum cells, monocytes, monocytoïd lymphocytes and lymphocytes. That a tumor of the lymphosarcoma group may contain several types of cells is well known. Ehrlich and Gerber¹⁷ showed that the tumor often starts as reticulum cell sarcoma and ends as lymphosarcoma. The finding of a pure growth of reticulum cells in the small axillary lymph node and the demonstration of a gradual transition from reticulum cells at the periphery to monocytoïd lymphocytes in the central portions of the larger tumor in this case tend to confirm their findings.

Of chief interest here, however, were the monocytoïd lymphocytes. Had they been studied only in the fixed preparation, they would have been classified as ordinary lymphocytes. In the supravital stained films they were seen to contain many more neutral red bodies than ordinary lymphocytes. Whether tumors composed of monocytoïd lymphocytes should be considered identical with tumors made up of ordinary lymphocytes cannot be determined until a large series of such tumors has been studied; in the meantime it seems worth while to keep these tumors segregated according to the supravital staining characteristics of their cells.

CASE 3.—A 58 year old Chinese man was first seen on Oct. 12, 1940 because of a large tumor mass which had been present in the right side of the neck for seventeen months. It grew slowly until it reached the size of a hen's egg at the end of fifteen months, but in the next two months it suddenly increased in size, and this growth was accompanied by attacks of pain and a sense of distention.

Examination revealed a tumor measuring 16 by 12 cm. in the right side of the neck. It was not tender; it was rubbery and fixed to the surrounding tissue. The overlying skin was stretched and reddened and showed a few small ulcers. The trachea was deviated to the left. Other findings were not important.

On October 23 a punch biopsy was made for histologic diagnosis. Roentgen radiation to a total dose of 2,000 r was given from October 28 to November 26. The original tumor showed marked regression, but new tumors developed in the left side of the neck and in the axillas during the period of treatment. The prognosis was considered grave, and further therapy was not attempted. A node, 1.8 cm. in diameter, from the left supraclavicular region (which had not been irradiated) was removed on December 5 for histologic study.

The blood findings were: red blood cells, 4,800,000; white blood cells, 9,950 per cubic millimeter; hemoglobin, 14.6 Gm. per hundred cubic centimeters. The differential count showed neutrophils 69 per cent, lymphocytes 21 per cent, monocytes 9 per cent and eosinophils 1 per cent.

Examination of the Tumor Tissue.—Sections prepared from the specimen removed by punch biopsy from the large tumor on October 23 and from the supra-

clavicular node obtained on December 5 and stained with hematoxylin and eosin showed a diffuse growth of large and medium-sized lymphoid cells with rounded, moderately hyperchromatic nuclei and a small to moderate amount of well stained cytoplasm with distinct cell borders (fig. 5 *B*). Among these cells were many

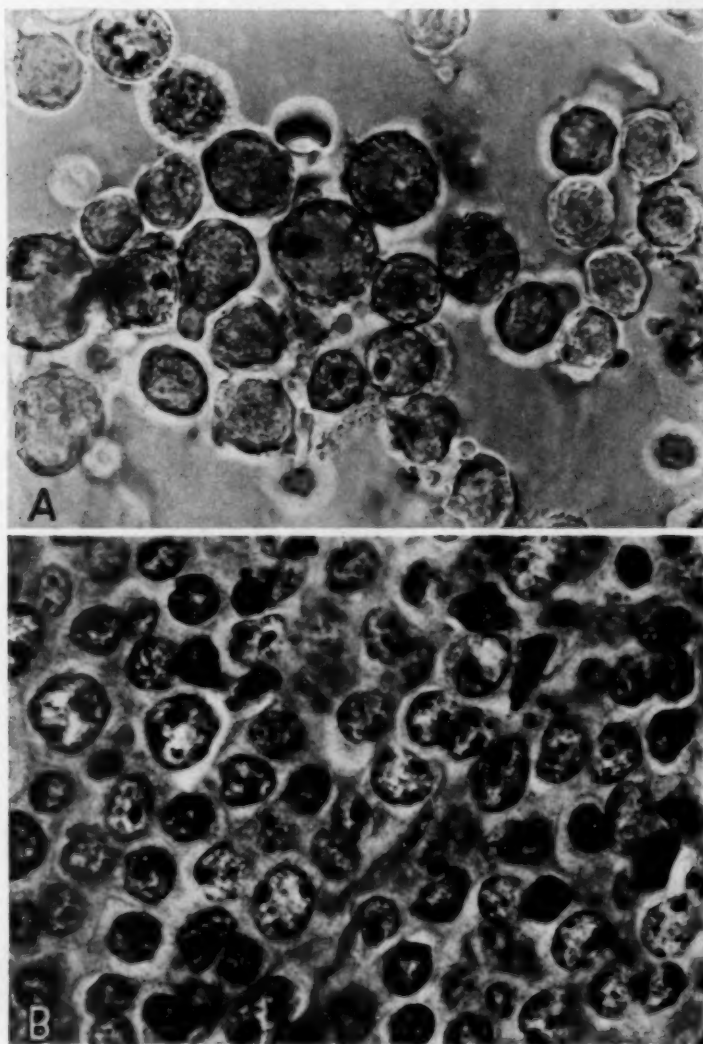


Fig. 5 (case 3).—Large and medium-sized tumor cells from the node in the left supraclavicular region. Note the round nuclei, the scanty cytoplasm and the absence or scantiness of the neutral red bodies. The dark color of the nuclear membrane of certain cells is due to staining by the neutral red and indicates death of these cells. Supravital staining; $\times 1,000$. *B*, tumor cells from the node in the left supraclavicular region showing large and medium-sized lymphoid cells with hyperchromatic nuclei and reticulum cells with large vesicular nuclei. Hematoxylin and eosin; $\times 1,000$.

reticulum cells with large rounded vesicular nuclei and pale, poorly defined cytoplasm. Between the well differentiated lymphoid cells and the reticulum cells many intermediate forms were found. Mitotic figures were numerous among the tumor cells. There also were areas of necrosis.

Supravital study was made of the tumor cells of the node removed from the left supraclavicular region. The cells were obtained by gently scraping the freshly cut surface with a cataract knife as in case 2. In the supravitaly stained film (fig. 5 *A*) one found that most of the cells had large or medium-sized rounded nuclei. Some of them were reticulum cells, which either had indefinite cytoplasmic outlines or had sharply outlined cell borders without stainable particles in the cytoplasm. Others were large and medium-sized lymphoid cells with rounded nuclei and scanty but rather opaque cytoplasm containing mitochondria but usually no neutral red bodies or only a very few fine red dots. A number of cells had their nuclei (especially the nuclear membrane) heavily stained by neutral red, indicating cell death. Monocytes or monocytoïd lymphocytes were practically entirely absent.

The oxidase reaction of the tumor cells was negative.

The diagnosis was lymphosarcoma (large lymphocytic type).

The principal tumor cells in this case were large lymphocytes, derived from the primitive reticulum cells. In contrast to the cells of the two previous cases, the cells in this instance contained very few neutral red bodies, and there was no congregation of such bodies or formation of rosettes.

If one compares the section of the fixed tissue in this case (fig. 5 *B*) with that in the previous case (fig. 4), one can note only a slight difference. But no one can consider the difference between the supravitaly stained cells of these two cases (figs. 5 *A* and 3 *A*) as slight, whatever the significance of this difference may be.

COMMENT

It is not the purpose of this paper to enter into the consideration of whether or not the monocytes represent a separate, independent strain of cells, whether or not the monocytoïd lymphocytes are merely a variety of the lymphocytes or whether or not the finer differentiation of the tumor cells and the diagnosis of the tumors as given in the case reports are justified. The meagerness of our material does not permit intelligent or helpful discussion of these problems, which can be profitably considered only after a large group of cases has been studied. Its principal object is to point out the fact that by the use of the supravital staining technic the characteristics of certain tumor cells, insignificant or unobservable in ordinary preparations, can be strikingly brought out, so that a finer differentiation can be made. An analysis of observations on a large number of tumors prepared by the supravital staining technic might therefore be expected to yield valuable information.

It does not seem reasonable to believe that the different types of tumor cell which have been observed in these 3 cases represent all the

types that may be found in tumors of the lymphosarcoma group. There are, for example, the clasmatoocytes, or histiocytes, which also are derived from reticulum cells and which may form tumors that have been classified as reticulum cell sarcoma, just as the tumors diagnosable as monocytic sarcoma have been in the past.

The tumors of bone marrow also should be studied with the supravital staining technic, since certain features of the myeloid cells are more sharply brought out by the supravital staining method.

SUMMARY

The cells of three tumors of the lymphosarcoma group were studied by means of the supravital staining technic in conjunction with the ordinary histologic methods. By the use of this technic certain features of the tumor cells, inconspicuous or unnoticeable in the ordinary microscopic sections, are rendered prominent. It is therefore suggested that this technic be used in combination with the ordinary histologic methods in the study of tumors of the lymph nodes and bone marrow.

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THE SPLEEN IN THE LEUKEMIAS

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AND

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PHILADELPHIA

Though the concept of leukemia has been before the medical public for almost a century,¹ the classification of leukemia into subvarieties according to essential natures still remains sub judice. As in the case of numerous other diseases, too, many statements as to symptoms, size of organs, range of blood counts and so on have been carried over from times when different concepts, based on less complete information, prevailed. It seemed therefore desirable to review such items in the light of recent findings in cases in which autopsy had provided a completed picture. Autopsies are none too frequent in true cases of leukemia, so that collection of adequate material, though mainly from our own records, has been a painstaking matter involving the records of a number of hospitals.² It goes without saying that reports coming from many clinicians and pathologists necessarily vary in completeness, accuracy and points of view, and though the material has been checked and the original material reexamined as far as possible, to produce uniformity of values, it is obvious that the study has suffered from these elements of vicarious research.

From the Department of Pathology, University of Pennsylvania School of Medicine.

Though this study has been in progress for over two years, it long since became apparent that it could not be properly completed in time for this special number in honor of Dr. Opie. We have therefore confined our attention in this article to an analysis of the findings on the spleen. We hope to publish the rest of the study later. The spleen is the best single organ to study in leukemia, as it is one of the most significantly altered organs in all types of leukemia. Its size also is most easily followed during life; even its components may be studied during life, by splenic puncture, if so desired.

1. Bennett, J. H.: *Edinburgh M. & S. J.* **64**:413, 1845. Virchow, R.: *Med. Ztg.* **15**:157 and 163, 1846.

2. The authorities of the following hospitals gave us permission to use their records: the Allentown, Pa., Lancaster, Pa., Lincoln, Neb., and Philadelphia general hospitals and the Allegheny, the Children's, Magee, Mercy and Western Pennsylvania hospitals of Pittsburgh. The following third year students in this school painstakingly examined and tabulated the material: Messrs. G. H. Amsterdam, K. P. Bachman, O. E. Baum, H. S. Belmont, R. B. Chodos, Miller, R. A. Rupp and J. E. Snyder.

Accepting for practical purposes that lymphoblasts, myeloblasts and monoblasts are distinct cells of independent origin, it has seemed best to subdivide the material into acute and chronic lymphatic (lymphogenous), acute and chronic myelogenous, and monocytic (combined acute and chronic) varieties, with as small a group as possible of unclassified forms. Examples of eosinophilic, basophilic and plasma cell leukemia were not encountered, and it is doubtful if these should be regarded as leukemia. Schridde's and Hirschfeld's classification into leukemic, subleukemic and aleukemic varieties is obviously of clinical rather than scientific value. The very difficulties of classifying acute forms, which have led many to stop at the diagnosis "acute leukemia," have challenged us to subdivide such leukemic conditions as far as legitimate into their histogenetic types in the hope of acquiring illumination of their true nature. We have changed the original pathologic diagnosis when the evidence warranted it. It was a pleasant surprise to find that further division of the acute types was possible in the great majority of cases.

"Subacute" leukemic conditions have had to be allocated to either acute or chronic categories. As "acute" and "chronic" are not true antonyms, it was not possible to set up rigid differentiating criteria. As regards chronicity, however, conditions of an apparent duration of more than four months were usually placed in the chronic class; as regards acuteness, the characteristic signs of an acute onset, a predominance of immature forms in blood and tissues and an absence of obviously longstanding tissue changes were taken as important evidence that the disease was acute, unless attributable for other reasons to exacerbation of a chronic course. The usually accepted criteria for distinguishing between lymphoblast, myeloblast and monoblast in tissues were applied according to taste; surely others would have decided differently in not a few cases. The criteria for allocating given cases to the chronic lymphatic and myelogenous varieties were, as a rule, easily applied. If a case was obviously one of lymphatic, or myelogenous, leukemia, predominance of mature over immature tissue cells usually sufficed for this purpose. The chief difficulty arose when the variety of leukemia was not otherwise indicated, and the cells both of peripheral blood and tissue infiltrations were of immature type. Even here, however, close study not infrequently revealed, in addition to the immature cells, more mature cells of a recognizable type that aided in identification. As regards the immature cells, by themselves they were often not differentiable, especially in routine hematoxylin-eosin tissue preparations; if, however, they tended to have large, round nuclei with finely divided (not coarse, as often

stated) chromatin and scanty, round neutrophilic cytoplasm, they were classified as lymphoblasts; if they had large, round, either dark-staining or relatively empty nuclei and usually a narrow zone of faintly basophilic cytoplasm, they were classified as myeloblasts (the presence of granules in oxydase preparations aided in the identification of cells of this series); if they had a round or indented nucleus with a moderate amount of chromatin, were pleomorphic and anisocytotic, with an irregular faintly acidophilic cytoplasm, tended to form streamers and were closely associated with a finely meshed tissue reticulum, they were taken to be monoblasts or monocytes, though there was but little opportunity to observe this type in detail. No attempt was made to differentiate subtypes on the basis of lymphoblast, lymphosarcoma cell and lymphocyte (Wiseman³).

MATERIAL

Our 209 cases of leukemia were drawn over periods of fifteen to twenty years from two general hospitals in this city (101 cases), from five hospitals in Pittsburgh, one a children's hospital (78 cases), from two general hospitals elsewhere in Pennsylvania and from one in Lincoln, Neb. (30 cases). Material with this geographic distribution should give a better view of leukemia in this country than material drawn from one source only.

INCIDENCE OF LEUKEMIA IN CASES IN WHICH AUTOPSIES WERE RECORDED

The incidence of the leukemia diagnosis in autopsied cases in these hospitals ranged from 0.33 per cent at "Blockley" to 1.85 per cent at the Children's Hospital of Pittsburgh. The hospital of the University of Pennsylvania, which has many referred patients with "blood diseases," had a percentage of 1.40; the average between this and the Blockley percentage—0.92—is probably close to the true hospital autopsy incidence of leukemia for this vicinity. The total leukemia incidence for 26,394 autopsies was 0.79 per cent.

The following types of leukemia were represented: acute lymphatic, 51 cases; chronic lymphatic, 48 cases; acute myelogenous, 44 cases; chronic myelogenous, 47 cases; monocytic, 7 cases, and unclassified, 12. It is obvious that these figures depend more or less on the personal judgment of the pathologists and also on that of the authors; however, with the better balance that results from a number of sources, these figures may be taken as fairly representative.

3. Wiseman, B. K.: J. A. M. A. **118**:100, 1942.

WEIGHT OF SPLEEN

In the routine weighing of spleens at autopsy, varying amounts of blood are lost. With respect to congested organs, the amounts may be considerable. The weight ranges and average weights of adult spleens in different types of leukemia in our series are given in table 1.

The spleens of children below 15 years of age were increased in weight with one exception. This was the spleen of a child of 33 months with acute lymphatic leukemia; it weighed 22 Gm. instead of a normal of about 55 Gm. Comparing the observed weights with those established for "normal" spleens at different ages (Krumbhaar and Lippincott⁴), we find that in 21 cases of acute lymphatic leukemia the child's spleen weighed on the average three times normal; in 3 cases of chronic lymphatic leukemia, six and four-tenths times normal; in 2 cases of acute myelogenous leukemia, six and nine-tenths times normal (163 and 610 Gm., the latter in a 30 month old child).

TABLE 1.—*Weight of Adult Spleen According to Type of Leukemia*

Type of Leukemia	Weight Range, Gm.	Average Weight, Gm.
Acute lymphatic.....	130 to 920	308
Chronic lymphatic.....	100 to 4,400	618
Acute myelogenous.....	86 to 1,650	428
Chronic myelogenous.....	160 to 4,930	1,686
Monoeytic.....	160 to 550	333

In table 2, the 180 cases in which the information was available have been distributed according to type of disease, sex, and weight of spleen. [In cases in which the patients were below 15 years of age, the spleen weights (enclosed in parentheses) have been allocated to appropriate columns of the adult spleen weight by corrections according to the normal spleen weight curve for the actual age.] It will be seen that, considering all types, the largest number of spleens fell in the 400 Gm. class, the second largest in the 300 Gm. and the third largest in the 200 Gm. class. Of the two spleens weighing more than 4 Kg., one was from a patient with chronic myelogenous, and the other from a patient with chronic lymphatic, leukemia. Males had significantly heavier spleens than females in chronic lymphatic leukemia; with other types sex seemed to make no great difference.

In Amano's⁵ somewhat smaller series, the average weights of the spleens of adults with the different types of leukemia were as follows: acute lymphatic (6 adults), 450 Gm.; chronic lymphatic (2 adults), 175 Gm.; acute myelogenous (22 adults), 225 Gm.; chronic myelogenous (17 adults), 1,651 Gm.

4. Krumbhaar, E. B., and Lippincott, S. W.: *Am. J. M. Sc.* **197**:344, 1939.

5. Amano, S.: *Jap. J. M. Sc., V., Path.* **5**:331, 1940.

TABLE 2.—*Distribution of Spleen Weight According to Type of Leukemia and Sex**

Type of Leukemia	Sex	Spleens in Given Weight Class															Sex Total	Type Total
		60-99 Gm.	100-199 Gm.	200-299 Gm.	300-399 Gm.	400-499 Gm.	500-599 Gm.	600-699 Gm.	700-799 Gm.	800-899 Gm.	900-999 Gm.	1,000- 1,499 Gm.	1,500- 1,999 Gm.	2,000- 2,999 Gm.	3,000- 3,999 Gm.	4,000- 4,999 Gm.		
Acute lymphatic.....	M	..	1+ (1)	2+ (1)	3+ (3)	5+ (3)	(2)	1+ (1)	..	1	2	(1)	14+ (12)	} 17+ (30) = 37
	F	(1)	(2)	1	1	1+ (3)	(1)	(1)	3+ (8)	
Chronic lymphatic.....	M	..	3	3	1	2	4	..	5	2	(1)	3	3+ (1)	2	..	1	29+ (2)	} 38+ (3) = 41
	F	..	2	1+ (1)	2	2	..	2	9+ (1)	
Acute myelogenous.....	M	1	..	5	3	1	2	2	1	..	1	..	1+ (1)	17+ (1)	} 34+ (3) = 36
	F	..	2	5	2	3	2	1	1	1	(1)	17+ (1)	
Chronic myelogenous.....	M	1	1	1	1	4	5	10	..	1	24	} 41
	F	..	1	..	1	3	1	..	1	1	..	4	2	1	2	..	17	
Monocytic.....	M	..	1	..	3	1	5	} 7
	F	1	1	2	
Atypical.....	M	..	1	3+ (1)	(1)	(1)	1	1	..	1	1+ (1)	8+ (4)	12
Total.....		3	14	23	20	26	14	8	9	6	5	14	15	13	2	2	174	

* Spleens from persons under 15 years of age are corrected for age and indicated with parentheses.

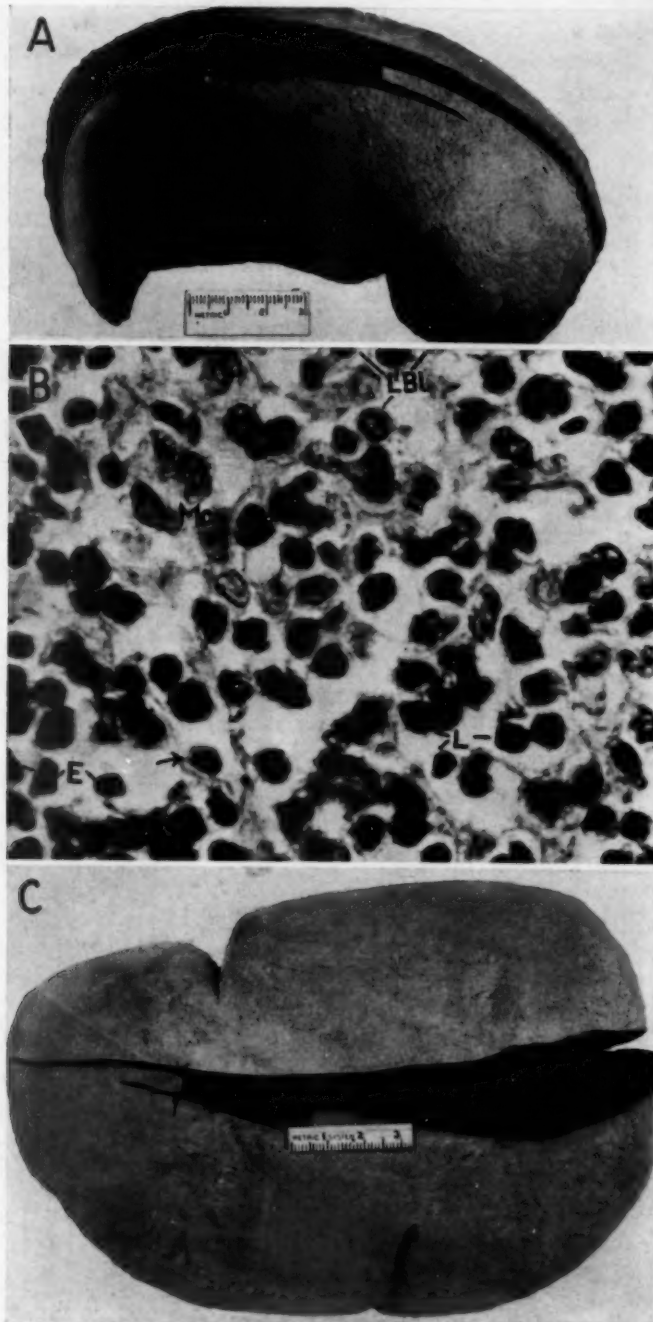


Figure 1

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CONDITION OF THE SPLEEN IN DIFFERENT TYPES OF LEUKEMIA

The following analysis is based on the data from 100 cases only (from the University Hospital, the Philadelphia General Hospital and the Lincoln, Neb., General Hospital); individual items of the analysis, however, were unfortunately recorded even here in considerably fewer instances than might be expected. Statistical analysis of the descriptions is prevented not only by their brevity but also by the unavoidable tendency to mention only the more striking positive observations; e. g., bulging of a cut surface is more apt to be recorded than absence of bulging.

In general, it may be said that in most items analyzed there was considerably more variation than would be expected from a study of the literature. As exceptions, when accessory spleens were found and studied, they were invariably found to present the same picture as the main organ; the lymph node follicles also generally, but not always, showed the same changes as the malpighian follicles of the spleen.

Pale centers of the follicles (the so-called germinal centers) were occasionally found in all types of leukemia. However, from the appearance of the cell and associated extracellular material they appeared to be cells of tissue reaction, not of increased hemopoiesis.

Infarcts were reported in 15 of 93 cases (15.7 per cent)—in 1 of 18 cases of the acute lymphatic type, in 3 of 25 cases of the chronic lymphatic type, in 1 of 22 cases of the acute myelogenous type, in 9 of 27 cases of the chronic myelogenous type, and in 1 of 4 cases of the monocytic type.

Acute Lymphatic Leukemia.—The capsule was reported as either normal or having minor thickenings. The consistency of the spleen was reported as firm almost as often as soft. The color of the cut surface was usually noted as grayish or dark red, but light red, light brown, reddish purple and slatish were also observed.⁶ Mottling was noted in only a few cases. No resistance to cutting and only rare bulging of the cut surface were recorded. To our surprise, the malpighian

6. The color of the unopened organ was not considered, as differences are due largely to the state of the capsule. Here, too, the variation in color sense and in descriptive power of the observers is an added handicap.

EXPLANATION OF FIGURE 1

A, spleen weighing 410 Gm. from a boy of 4 with acute lymphatic leukemia (U. P. '28-25). Note the slightly mottled bulging cut surface and the thin capsule, wrinkled by shrinkage and loss of blood from the organ.

B, high power magnification ($\times 538$) of spleen of acute lymphatic leukemia (U. P. '41-1251). Most of the cells are lymphocytes (*L*), some showing immaturity (*LBI*). There are a few erythrocytes (*E*) and monocytes (*Mo*).

C, enlarged spleen, weighing 850 Gm., from a man of 42, with chronic lymphatic leukemia (U. P. '29-151), showing increased thickness, two deep notches, small capsular thickenings and a cut surface with markings preserved.

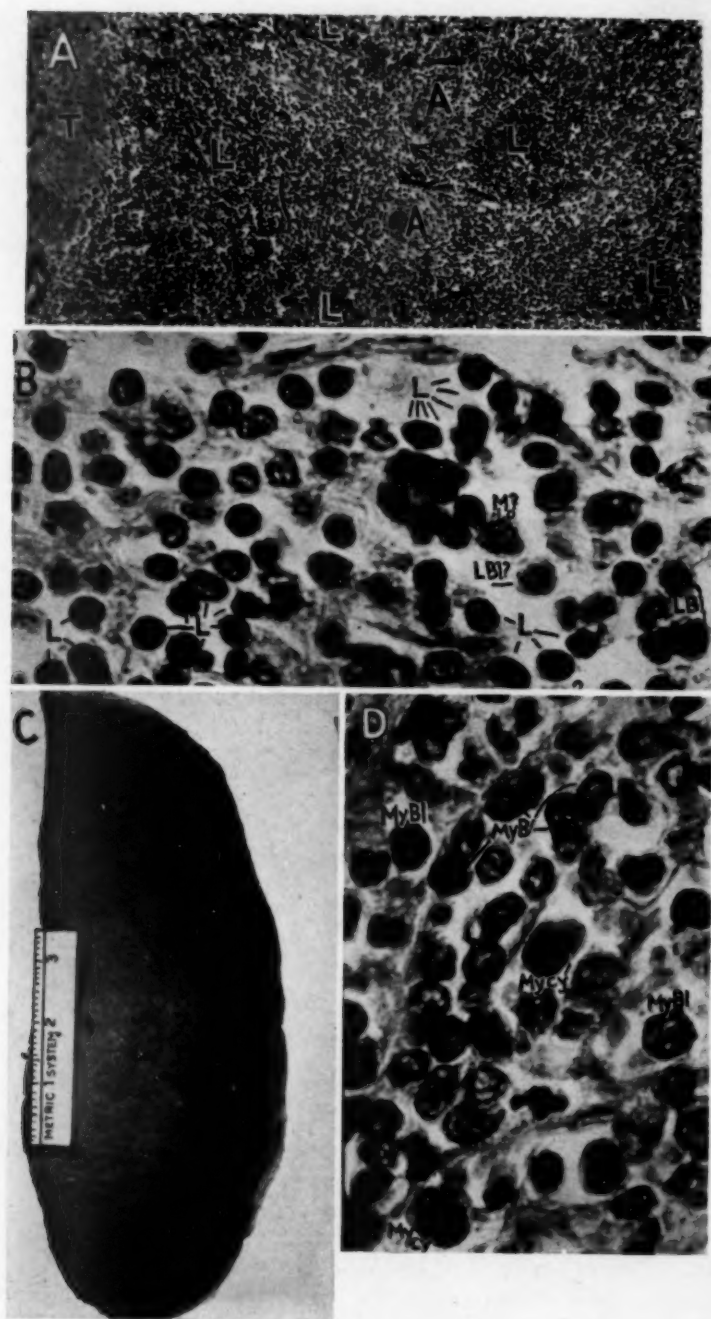


Figure 2

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follicles were reported grossly and microscopically in about equal numbers of cases as absent, small, indistinct or prominent. However, on review these findings are all compatible with the impression that the lymphocytes of both the follicles and the pulp are almost always increased in lymphatic leukemia; if the infiltration of the pulp is less marked, the follicles appear prominent; if it is more marked, the follicles may appear large or small but indistinct; if it is very marked, the structural pattern, including the follicles, may be obliterated. Infiltration of the splenic pulp by the leukemic cells was almost always observed, and it was usually noted that these cells were of the large, immature type of lymphocyte. Occasional protocol statements as to increase of trabeculae or reticulum on gross inspection were regarded as relating to alterations due to old age or other disease. One small, old infarct of the spleen was observed.

Chronic Lymphatic Leukemia.—The capsule was usually found to be normal, though thickenings were noted seven times and adhesions three times. The consistency was reported as soft six times and firm ten times. The color ranged through various shades of red, with one capsule slatish and several mottled. The malpighian follicles were reported as rarely absent, usually small, rarely "large and prominent." Figure 2A shows how the barely recognizable malpighian follicles may at times be actually more sparsely populated (exhausted factories?) with lymphocytes than the surrounding pulp. Pulp infiltration by lymphocytes was almost always noted, with small, adult cells predominating and giant cells present occasionally. Trabeculae and reticulum were noted as increased, normal or decreased in about equal proportions. The general structural pattern was frequently found to be changed or even obliterated.

Acute Myelogenous Leukemia.—Only four times was a thickening of the capsule noted, hemorrhage once and adhesions not at all. The organ was more apt to be soft than firm (13:4). The color was purple four times, red six, red-gray six and pale red four times. The cut surface tended to bulge. The malpighian follicles were never enlarged, rarely normal, usually small or few and rarely indistinct or absent. Chiefly immature myeloid cells infiltrated the pulp, with fewer myelocytes and neutrophils. The usual difficulty in differentiating between lymphoblasts, monoblasts and myeloblasts was frequently referred to.

Chronic Myelogenous Leukemia.—The capsule showed thickenings or adhesions about as often as it was reported normal. Various degrees of firmness were

EXPLANATION OF FIGURE 2

A, splenic tissue ($\times 61$) in a case of chronic lymphatic leukemia (U. P. '40-1025), showing marked infiltration of the pulp by lymphocytes (L). Near the center are two follicular arteries (A) in a relatively empty follicle, the area of which is roughly outlined by the more densely infiltrated pulp. A trabecula (T) appears at the left.

B, high power magnification of A ($\times 657$). There is a marked predominance of adult lymphocytes (L), though lymphoblasts (Lb1) and other types of leukocytes are also to be found. Mo? indicates what is probably a monocyte.

C, slightly enlarged spleen (163 Gm.) from a girl of 6 with acute myelogenous leukemia (U. P. '36-1386). Note the pigskin effect from postmortem dimpling of the capsule, which has retained spots of blood clot, deposited at autopsy.

D, hematoxylin-eosin preparation ($\times 657$) of splenic tissue in a case of acute myelogenous leukemia (U. P. '36-23). Note the predominance of immature myeloid cells (MyB1) with occasional granular myelocytes (MyCy).

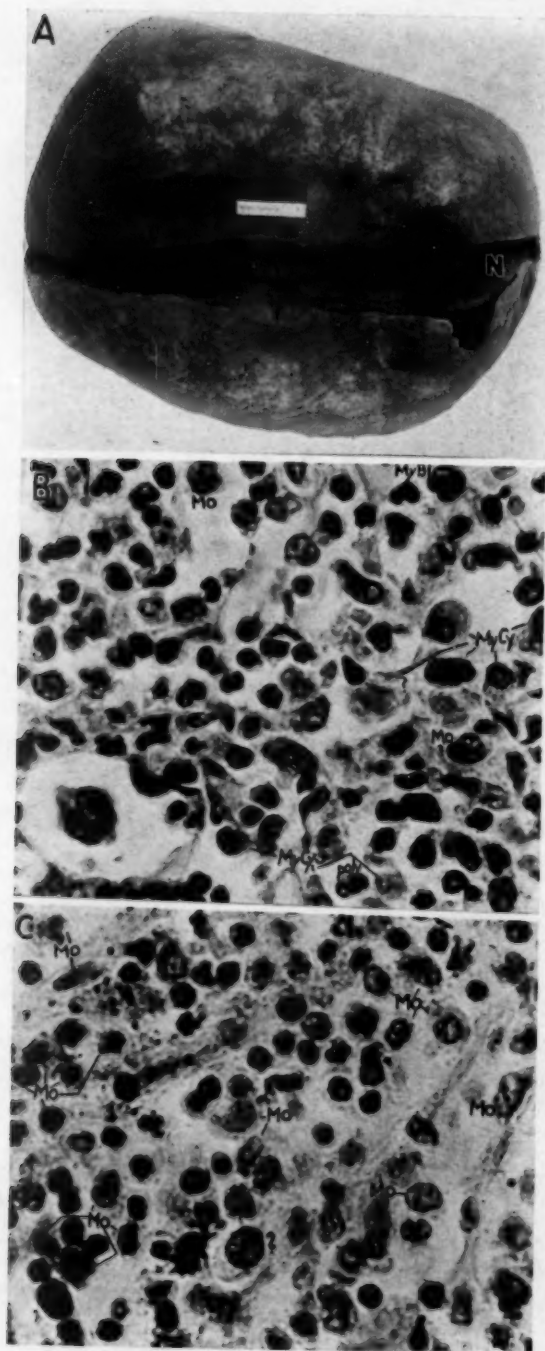


Figure 3

(See legend on opposite page)

noted about twice as often as softness, with occasional notation of resistance to cutting. The cut surface was occasionally noted as bulging. The usual range of color was observed, the reds predominating, but more slates and gray-blues than usually recorded in leukemic spleens, and once "bacony" was recorded. Malpighian follicles, as was to be expected, were reported as small or absent more frequently than in the other types, and in no case as large or prominent. Pale centers, such as might have been caused by masses of myeloid cells as well as by a reactive process, were apparently not seen. The red pulp was regularly infiltrated with myeloid cells. Giant cells, megakaryocytes and erythroblasts, suggestive of metaplasia, were occasionally found, though far less often than was to be expected from the literature on the subject. This was also true of prominent notching of the spleen and of fibrosis of its pulp. The marked fibrosis stressed by some authors was seldom recorded.

Monocytic Leukemia.—Available details of this type were too few (4 cases) to give a sound basis for evaluation, especially as it is a variety about which there is at best much difference of opinion. It was not thought to be worth while making a division into acute and chronic forms; in 3 instances the condition was of less than four months' duration. Three of the spleens were soft, and one was of rubbery consistency. They were paler than the others; three were gray-red and one was gray-pink. The capsules of all were normal. The follicles were noted as "not prominent" three times and absent even on microscopic examination once. Pale centers were noted as absent once and slightly increased once. (In this type of leukemia the latter change might be due either to a reactive process or to a leukemia increase of the reticuloendothelial cells regularly found in these centers.) The pulp was uniformly infiltrated with cells taken to be of the monocytic variety, occasionally with some lymphocytes, eosinophils and myelocytes. A very intimate relation to a finely meshed reticulum was observed in specially stained tissues. The splenic structure was noted as normal twice; the trabeculae, as diminished twice and increased twice.

SOME CASES ILLUSTRATING EXTREMES IN SPLEEN WEIGHTS

[NOTE.—Table 1, in which a wider range is given, includes cases from hospitals where splenic details were not available.]

Acute Lymphatic Leukemia.—One of the lightest noninfantile spleens of this type—weight 150 (170, corrected) Gm.—was from a Russian boy of 13 years (U. P. '26,740), who for one month had suffered from increasing weakness and anemia, with bleeding from the nose and gums and petechiae. His blood leukocyte

EXPLANATION OF FIGURE 3

A, greatly enlarged spleen (1,900 Gm.) from a man of 59 with chronic myelogenous leukemia (U. P. '26-699). Note the varying thickness of the capsule and an area of necrosis (*N*).

B, high power magnification of tissue ($\times 492$) from spleen shown in *A*, revealing more mature (*MyCy*), but also immature (*MyBl*; *poly*), myeloid cells than in figure 2*D*. Note the megakaryocyte in a sinus at the lower left.

C, high power magnification of tissue from the spleen in a case of monocytic leukemia ($\times 492$). Pleomorphism of the monocytes (*Mo*) is only moderately well shown. In this field numerous other cells are present. The character of the questioned cells is in doubt.

count was 3,900 (neutrophils, 1 per cent; lymphocytes, 96 per cent; monocytes, 3 per cent). Four per cent of the leukocytes were oxydase positive. There were marked anemia and thrombopenia. The spleen was dusky gray and had a normally thin capsule, the pulp showed mild congestion and marked infiltration by immature lymphocytes, which greatly obscured, even microscopically, the outlines of the malpighian follicles. Faint, small "pale centres" were occasionally found. The lymphoblasts were distinguished, with difficulty, from myeloblasts and monoblasts by their large, finely divided round nuclei, smooth neutrophilic cytoplasm and association with adult lymphocytes in the absence of adult myelocytes and monocytes.

The heaviest spleen in this group—920 Gm.—was from a boy of 14 (U. P. '38-20), who had been ill for six weeks with fever and bleeding. The leukocyte count was only 7,000 (78 per cent lymphocytes, 6 per cent lymphoblasts). Many immature lymphocytes were found in the liver and in the enlarged cervical, inguinal, axillary and peribronchial nodes. The spleen was of normal shape and moderately firm; the cut surface was light red. The follicles were very indistinct, and the structural pattern was generally altered by the infiltrating immature lymphocytes. No cause for the unusually great enlargement was ascertained.

Chronic Lymphatic Leukemia.—In a white woman of 40 (U. P. '33-192), who for over two years had had progressive weakness, dyspnea and anemia, fever and bleeding from the gums developed a few weeks before her death in delirium. Lymphopoiesis and also erythropoiesis were prominent in the bone marrow, the liver and the spleen, which weighed only 155 Gm. Her leukocyte counts were 2,800 and 4,700 (due to neutropenia). She died of pneumonia.

A white man of 20 (U. P. '26-541), who had been known to have leukemia for at least ten months, had a leukocyte count of 243,000 (90 per cent lymphocytes, nongranular by oxidase stain). The greatly enlarged lymph nodes and liver were packed with adult lymphocytes, as was the spleen, which weighed 4,400 Gm. It showed two anemic infarcts, and the capsule had many adhesions. The malpighian follicles were totally obscured grossly and microscopically because of the massive cellular infiltration and marked patches of congestion. No special reasons for the great enlargement were revealed in the study of this case.

Acute Myelogenous Leukemia.—A white woman of 72 (P. G. H. 32,037) had been acutely ill for three weeks with weakness, stupor and fever, with a leukocyte count of 3,400 (74 per cent were of the myeloid series in various stages of immaturity; 12 per cent, "monocytes"). The pale red spleen weighed only 130 Gm. and was fibrotic. The small dark malpighian follicles, without pale centers, stood out microscopically against the paler staining pulp, which contained predominantly immature myeloid cells with large relatively empty nuclei and nongranular, slightly basophilic nuclei.

On the other hand, the spleen of a white man of 35 (U. P. '38-1178), who had been ill for eleven weeks, weighed 900 Gm. It had a bright red bulging surface with indistinct markings. The peripheral blood was aleukemic, but myeloid cells in increased numbers and various stages of immaturity were found in the bone marrow, enlarged lymph nodes, liver and spleen.

Chronic Myelogenous Leukemia.—The lightest adult spleen (Lincoln G. H.)—160 Gm.—was from a white man of 70, who had been ill for two years. His leukocyte count had never been high, the last one being 3,600 (68 per cent of which were myelocytes and myeloblasts). He had enlargement of the cervical nodes and much bone marrow hyperplasia but no cell infiltrations in the liver. The splenic structure was fairly well preserved, with persisting follicles with pale centers. Many myelocytes, however, were recognized through the pulp. This

is obviously a valid example of chronic myelogenous leukemia of mild myelopoietic ability, with late hemorrhages and a fatal outcome in spite of an apparently mild course.

The heaviest spleen—4,930 Gm.—was from a white man of 40 (U. P.'35-32) whose blood count reached 191,000 leukocytes (14 per cent neutrophils, 81 per cent immature myelocytes). The bone marrow showed excessive myelopoiesis; the liver and various chains of lymph nodes were enlarged by myelogenous cells in all stages. The firm mottled red and yellow-gray spleen showed marked obliteration of structure by the infiltrating myeloid cells. As usual, it was not apparent why the spleen was so much heavier than in other similar cases.

Monocytic.—The spleen of a white man of 60, who had been ill six months, with a leukocyte count ranging between 52,000 and 67,000 (neutrophils, 20 per cent; lymphocytes, 20 per cent; monocytes, 60 per cent), weighed at autopsy 410 Gm. (U. P. '30-909). The cut surface was gray-red, with the capsule thin and smooth and the malpighian follicles small. Microscopically, however, they were easily distinguished from the lighter staining pulp, which contained predominantly pleomorphic cells, varying greatly in size, with moderately dark nuclei, often indented and with prominent nucleoli. The cytoplasm of these cells tended to be slightly acidophilic and angular, and the cells lay in close connection with a finely meshed reticulum. Stout trabeculae were conspicuous and adult lymphocytes not uncommon.

Thus, while the atrophy of old age and the low blood leukocyte count appear to be factors in producing some light spleens, explanations for both extremely low and high weights are generally lacking.

COMMENT

It is so obviously preferable to consider the leukemias in a classification of acute and chronic varieties of their histogenetic types that we merely urge that if writers on the subject feel it necessary to classify on some such basis as leukemic, subleukemic and aleukemic varieties, they at least include in their nomenclature the cell type involved.

In our series, cases were distributed more evenly among the four main types than is found in the literature. We have omitted from our classification the localizing neoplasms (lymphosarcoma, myeloma, chloroma and so on); likewise, the eosinophil, basophil and plasma cell varieties, both because cases of these types are very rare (we had none in our series) and because we share in the opinion that these are very probably atypical variants rather than basic types of leukemia. The differentiation of leukemoid conditions⁷ from true leukemia, which Apitz⁸ has stated is one of the important tasks remaining for those who are working on the problems of leukemia, had best be left for our later paper, which will be of broader scope. This problem has been a minor vexation in the present study in a few cases of lymphosarcoma, atypical tuberculosis, agranulocytosis, aleukemic reticuloendotheliosis and so on.

7. Krumbhaar, E. B.: *Am. J. M. Sc.* **172**:519, 1926.

8. Apitz, K.: *Ergebn. d. allg. Path. u. path. Anat.* **35**:1, 1940.

Against the not infrequent contentment with the diagnosis "acute leukemia," we submit that in a reasonably complete study of a case the differentiation into the proper type can regularly, if not constantly, be made with no more difficulty than may be encountered in differentiating between acute and chronic forms of a given type. In the latter problem, an obscure onset with the possibility of a late exacerbation, prominent in symptoms and tissue changes, was at times a real obstacle, in that it masked what was really a chronic process. It appears, then, that the fact that the diagnosis may not have been possible during life is not sufficient ground for avoiding the classification of acute forms in their several types, for purposes of comprehension and scientific progress.

Though some of the malpighian follicles have been reported as large and prominent, we have encountered only 1 case in which the question of giant follicular hyperplasia arose. In this case, in which giant follicular hyperplasia had been found in an inguinal lymph node five years before death, the spleen, like the involved tissues generally, at autopsy showed the lesions of lymphosarcoma. We regard this as strengthening the view that giant follicular hyperplasia—hardly a concise entity at best—is but a preliminary stage in the development of lymphatic leukemia or lymphosarcoma.

Death during an aleukemic phase of the disease—a not infrequent occurrence, as will be seen in our later study—was expected a priori to be associated with a relatively smaller spleen. This, to be sure, was frequently found, as in the case of a 3 year old boy suffering from acute lymphatic leukemia with a leukocyte count of 4,100, who had a spleen weighing 22 Gm.—only four tenths of the normal weight for that age. On the other hand, it was often found that the opposite was true; e. g., another 3 year old patient with acute lymphatic leukemia and a leukocyte count of 3,700 had a spleen weighing 200 Gm., three and six-tenths times the normal weight for that age. This is but another example of the wide range of variation in different cases of the same type of leukemia, and of the danger of generalizing on theoretic or on slender factual bases.

Though modern biologic methods of cytologic investigation (e. g., mode of locomotion, tissue cultures, vital staining) appear to have demonstrated significant changes from the normal to the neoplastic lymphocyte (the Lewises⁹; Richter and McDowell¹⁰), such studies have not as yet shown convincing differences between the lymphocyte of lymphatic leukemia and that of lymphosarcoma. The classic histologic method also often fails to afford satisfactory differentiation of these two conditions. To be sure, the lymphosarcomatous lesion may be more localized and has some tendency to infiltrate the splenic capsule and

9. Lewis, W. H.: Tissue Culture in the Study of Cancer, in a Symposium on Cancer, Madison, Wis., University of Wisconsin Press, 1938, p. 101. Lewis, M. R., and Mider, G. B.: *J. Nat. Cancer Inst.* **2**:115, 1941.

10. Richter, M. N., and McDowell, E. C.: *Physiol. Rev.* **15**:509, 1935.

perisplenic tissue; this picture is rarely found in the spleen of lymphatic leukemia though present in the more delicate lymph node capsule. In late stages of diffuse splenic lymphosarcomatosis with obliteration of splenic structure the picture in one condition can easily be indistinguishable from that in the other. As in cases of lymphosarcoma the offending cells can in late stages spill into the blood stream, producing a leukemic blood picture—the condition known as leukosarcoma—the great overlap of lymphatic leukemia and lymphosarcoma is easily apparent.

With the wide acceptance of the neoplastic nature of the leukemic process, it seems reasonable to regard lymphatic leukemia and lymphosarcoma, myelocytic leukemia and myeloma, monocytic leukemia and retothelial sarcoma as but different expressions of neoplastic change of the cells in question. This, of course, need not imply that each member of each pair must have the same symptom complex, prognosis and treatment as the other, any more than in the case of different types of carcinoma arising from cells of the same type, such as carcinoma of the lip and carcinoma of the tongue. Concerning the objection that leukemia differs from lymphosarcoma in being a systemic disease, we submit that neoplastic change in cells, many of which are normally destined to reach the peripheral circulation, naturally can produce a systemic disease—an unprecise term at best—as well as a localized lesion if the neoplastic cells in question remain in the tissues until metastasis occurs in the classic cancer manner.

Concerning the weight of the spleen in the different types of leukemia, the most striking observation was the wide variation (much greater than the literature indicated) and the high average—779 Gm.—for all spleens. Here, however, it should not be overlooked that one spleen weighing over 2,000 Gm. will pull more than three spleens of 400 Gm. above this average; it seems more important that the three largest groups (in terms of adult spleens) were in the 200 to 500 Gm. classes, and in these groups as might be expected the acute varieties predominated. While most of the spleens from patients with chronic lymphatic leukemia fell in the 500 to 2,000 Gm. class, with one from a patient who undoubtedly had this type of leukemia weighing 4,400 Gm., the spleens from patients with chronic myelogenous leukemia were concentrated in the 1,000 to 2,999 Gm. classes, with the largest weighing 4,930 Gm. In connection with this type, spleens weighing over 5 Kg. have been not infrequently reported, and one is said to have weighed over 10 Kg. (MacCallum¹¹). Except for the well known fact that spleens representing the chronic leukemias tend to be heavier than those representing the acute leukemias, we were not able to establish even a rough proportionality between weight of spleen and length of process in individual cases.

11. MacCallum, W. G.: *Textbook of Pathology*, ed. 6, Philadelphia, W. B. Saunders Company, 1936, p. 893.

SUMMARY

An analysis was made of 209 cases of leukemia that were recorded with autopsy reports in nine hospitals in Pennsylvania and one in Nebraska. The cases of leukemia were divided as follows: acute lymphatic leukemia, 51 cases; chronic lymphatic, 48 cases; acute myelogenous, 44 cases; chronic myelogenous, 47 cases; monocytic, 7 cases; unclassified, 12 cases. This gives a total incidence of leukemia of 0.79 per cent in 26,394 autopsies.

In general, patients with the chronic forms have larger spleens than those with the acute, and those dying in aleukemic stages tend to have smaller spleens than those in whom marked leukocytosis persists to the end.

The splenic capsule was almost but not quite always normal in the acute varieties. In chronic lymphatic leukemia, the ratio of normality to thickening of the capsule to adhesions was 4:2:1; in chronic myelogenous leukemia, 4:3:1.

The color and the consistency of the spleen vary greatly but depend in good measure on the amount of congestion and leukemic infiltration rather than on the type found.

Malpighian follicles tend to be increased in earlier stages of the lymphatic forms. They become indistinct or vanish entirely in all forms when the pulp becomes sufficiently invaded by the leukemic cells. In the lymphatic forms, multiplication of lymphocytes occurs in both follicles and red pulp; in myelogenous forms, myelogenous cells are found multiplying in the pulp. Pale centers of the follicles (so-called germinal centers) were found only when there was a tissue reaction to excessive cell destruction (as in burns, diphtheria and other infectious diseases).

Fibrosis is generally regarded as commonest in the chronic myelogenous type; in our series, it was noted more often in the chronic lymphatic and occasionally even in the acute forms.

Infarcts were found in about 15 per cent of the cases—occurring in all types, but very rarely in the acute varieties and most commonly in the chronic myelogenous (9 of 27).

If one accepts the neoplastic nature of all the true leukemias, one can profitably regard lymphosarcoma as essentially the same disease as lymphatic leukemia, namely, a neoplasm of the lymphocyte. Lymphosarcoma manifests itself, at first, however, in the lymphatic tissues and may differ considerably in manifestations, prognosis and treatment from lymphatic leukemia, which is manifest first and predominantly in the peripheral blood. Each, however, in later stages frequently overlaps the picture of the other, giving rise to the picture in tissues and in peripheral blood conveniently known as leukosarcoma.

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TUMORS OF THE NERVE SHEATHS IN FISH OF THE SNAPPER FAMILY (LUTIANIDAE)

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The circumscribed tumors of peripheral nerves are a complex group of neoplasms which hitherto have been studied chiefly in man. Here they occur as solitary or multiple growths, which some observers regard as separate types, others as related variants. It is well known that these tumors superficially resemble neoplasms of connective tissue; they have, however, certain distinctive characters not found in other tumors. Thus they usually consist of two well defined types of tissue, the Antoni A and B types; they often, though inconstantly, form nuclear palisades, which are considered pathognomonic; their cells and intercellular fibers are frequently arranged as interlacing fasciculi. It is agreed that these tumors are derived from nerve sheaths, but whether primarily from the ectodermal schwannian component or from the mesodermal endoneurial-perineurial component is controversial. Reflecting opinions concerning their histogenesis, the tumors are called neurinoma, neurilemmoma, schwannoma, perineurial fibroma, neurofibroma and still other names; moreover, the concurrence of visceral involvement in some varieties has led to the recognition of syndromes, of which that of von Recklinghausen is the best known. Since the pertinent literature has been fully reviewed by several recent writers,¹ no further details need be given here.

In this paper I shall make no attempt to decide between opposing views about these tumors. Rather, I wish to present a different

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From the Laboratory of Pathology of the University of Pennsylvania School of Medicine, and the Tortugas Laboratory of the Carnegie Institution of Washington.

1. (a) Masson, P.: *Am. J. Path.* **8**:367 and 389, 1932. (b) Penfield, W.: *Cytology and Cellular Pathology of the Nervous System*, New York, Paul B. Hoeber, Inc., 1932, sect. 19. (c) Stout, A. P.: *Am. J. Cancer* **24**:751, 1935; **25**:1, 1935. (d) Geschickter, C. F.: *ibid.* **25**:377, 1935. (e) Foot, N. C.: *Am. J. Clin. Path.* **6**:1, 1936. (f) Worster-Drought, C.; Dickson, W. E. C., and McMenemey, W. H.: *Brain* **60**:85, 1937. (g) Bailey, P., and Hermann, J. D.: *Am. J. Path.* **14**:1, 1938. Tarlov, I. M.: *ibid.* **16**:33, 1940. (h) Ewing, J.: *Neoplastic Diseases*, ed. 4, Philadelphia, W. B. Saunders Company, 1940. (i) Murray, M. R., and Stout, A. P.: *Am. J. Path.* **16**:41, 1940. (j) Kernohan, J. W.: *Arch. Path.* **32**:843, 1941.

approach to their study, namely, that through comparative oncology. Tumors of the peripheral nerves have been found in widely different species: in cattle,² horses, mules, dogs and deer,³ in chickens,⁴ in a rabbit⁵ and in a goldfish.⁶ But in these species the tumors are relatively rare and hence not readily available for study. They are common, however, in certain marine fishes, for neoplasms of this kind have been found by me in 76 fish belonging to three species of snappers, the gray snapper (*Lutianidus griseus*), the dog snapper (*Lutianidus jocu*) and the schoolmaster (*Lutianidus apodus*). These are large fishes, averaging several pounds in weight; they are widely distributed in subtropical and tropical waters and are abundant in Florida and the West Indies. Most of the specimens on which this report is based were taken near the Dry Tortugas (a group of tiny islands in the Gulf of Mexico about 70 miles [112 kilometers] west of Key West); some were obtained near Key West. Descriptions of these fish and their habits will be found in the recent monograph by Longley and Hildebrand.⁷

The majority of the tumor-bearing fish were taken alive or had recently been killed. The tumors were fixed either in Bouin's or Susa fluid, or in 4 per cent solution of formaldehyde; paraffin served as embedding medium, and Masson's⁸ trichrome mixture and hematoxylin-eosin as routine stains; in addition, various silver stains were used.

This paper deals with the pathology of the fish tumors and with a comparison of these neoplasms with corresponding tumors of man.

PATHOLOGY OF THE TUMORS

Gross Appearance and Anatomic Distribution.—The appearance of representative tumors is shown in figures 1 and 2. They arise in the corium and project outward, stretching the covering epiderm to a delicate membrane devoid of scales. In shape they are oval or hemispherical with a flattened base. They range in size from small nodules to large masses as much as 5 cm. in diameter. Most of the tumors are

2. Frauchiger, E., and Hofmann, W.: *Die Nervenkrankheiten des Rindes*, Bern, Hans Huber, 1941.

3. Feldman, W. H.: *Neoplasms of Domesticated Animals*, Philadelphia, W. B. Saunders Company, 1932.

4. Jackson, C.: *Onderstepoort J. Vet. Sc. & Animal Industry* 6:3, 1936; *J. South African Vet. M. A.* 7:69, 1936.

5. Salaskin, B. A.: *Ztschr. f. Krebsforsch.* 30:371, 1930.

6. Picchi, L.: *Sperimentali* 86:cxxviii, 1933.

7. Longley, W. H., and Hildebrand, D. F.: *Systematic Catalogue of the Fishes of Tortugas, Florida, with Observations on Color, Habits and Local Distribution*, Publication 535, Carnegie Institution of Washington, 1941.

8. Masson, P.: *J. Tech. Methods* 12:75, 1929.

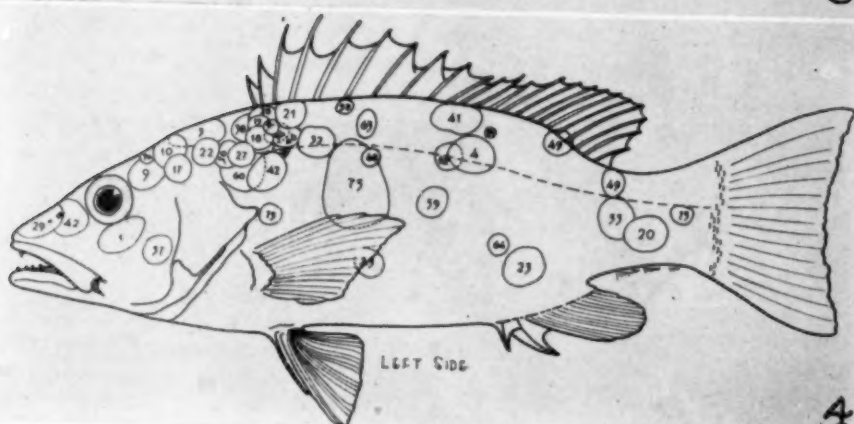
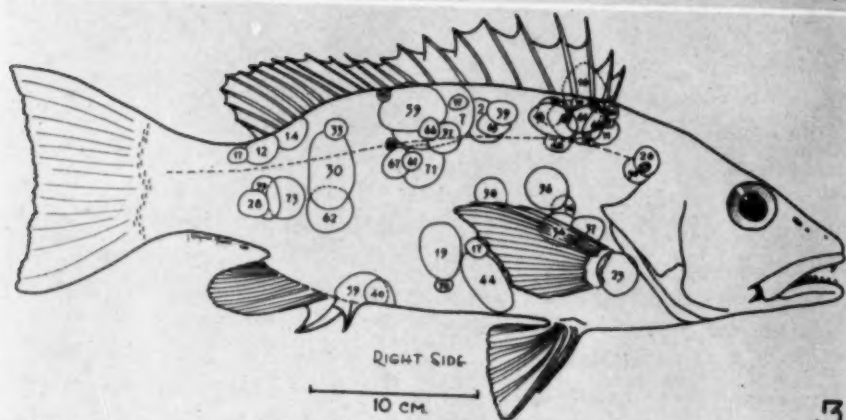
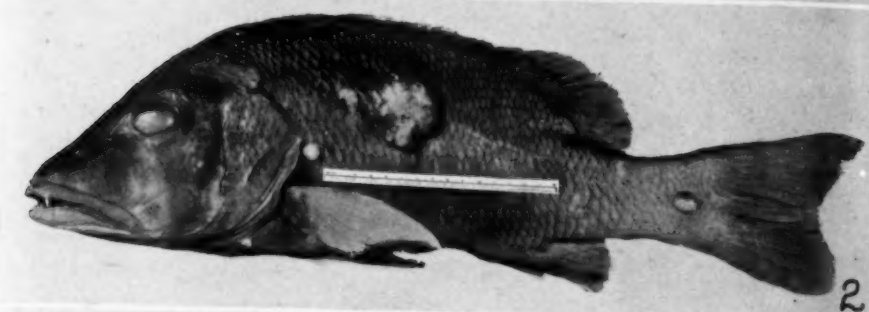
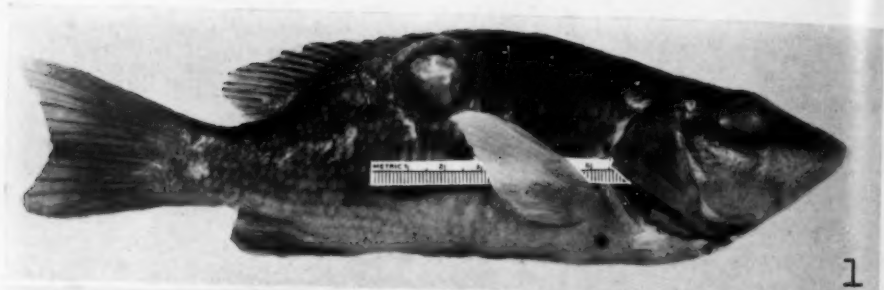
firm and resilient. Their cut surface is white, moist but not bloody, of nearly homogeneous texture in some areas, striated or whorled in others. All of the tumors are fairly well circumscribed, but none are enveloped by a definite capsule. At their base, they are usually sharply delimited, but some tumors infiltrate to a slight extent the subjacent muscles.

Usually only a single tumor is present, but two or rarely more may occur. In the present series the tumors were solitary in 65 fish; 7 fish had two tumors, 2 had three, and 2 others four or five tumors, respectively. The distribution of the tumors is graphically shown in figures 3 and 4. It is seen that certain definite areas of the body are commonly affected and others rarely. The majority of the tumors are located on the upper dorsal part of the fish, a particularly favorite site being the area just back of the head. A much smaller proportion involves the neighborhood of the pectoral fins or other areas on the lower half of the body; the ventral surface is affected in but a single case. This distribution corresponds to the course of the larger subcutaneous nerve trunks. The exact connection of tumors to nerves proved difficult to determine, for the subcutaneous nerves of fish are relatively small and abundant, and the tumors relatively large. While it is easy to trace nerves to tumors, it is difficult to be certain that the connection is other than fortuitous.

Histologic Appearance of the Tumors.—Before describing the structure of the neoplasm, it seems best to discuss briefly the relevant histologic aspects of the normal skin and of the peripheral nerves of these fishes.

The skin of snappers, as of all vertebrates, is made up of epiderm, corium and subcutaneous tissue.⁹ The epiderm consists of several layers of epithelial cells which rest on a coarse homogeneous basement membrane. The corium, with which this study is especially concerned, is much broader than the epiderm and is divisible into an outer loose-textured connective tissue, in which are embedded the bases of the scales, and a deeper layer, the stratum compactum. The outer layer carries an abundance of nerves, blood vessels and wandering cells; the deeper layer consists principally of closely packed parallel bundles of dense fibrous tissue and forms a tough boundary against the subcutaneous tissue (fig. 5). The latter is a relatively scanty fatty-areolar layer, in which lie the larger nerve trunks, branches of which extend outward into the skin and downward into the muscles.

9. Rauther, M.: *Echte Fische*, in Bronn, H. G.: *Klassen und Ordnungen des Tier-Reiches*, Leipzig, Akademische Verlagsgesellschaft, 1927, vol. 6, pt. 1. Krause, R.: *Mikroskopische Anatomie der Wirbeltiere*, in *Einzel Darstellungen*, Berlin, Walter de Gruyter & Co., 1923, vol. 4.



Figures 1 to 4
(See legends on opposite page)

The peripheral nerves of fish have the same general structure as mammalian nerves. They differ only in the somewhat larger size of some of the component parts.¹⁰

As to the histopathology¹¹ of the tumors, I shall discuss first the relation of the tumors to the neighboring structures. The relation to the subjacent tissue is of three different degrees (figs. 5 to 7). In approximately 60 per cent of this series the tumors are sharply delimited at their base by the stratum compactum (fig. 5). They arise in the loose portion of the corium and grow outward, expanding and compressing the tissue and destroying such readily recognizable components as scales. Externally, the tumors are usually limited by the epidermal basement membrane, against which are pressed fibrous remnants of the corium.

In a smaller proportion, approximately 30 per cent of the series, the tumors completely destroy the stratum compactum but do not invade the subcutaneous tissue (fig. 6). Ten per cent of the tumors extend beyond the subcutaneous tissue, infiltrating and destroying the superficial parts of the muscle (fig. 7). Such tumors also invade to some extent in a lateral direction. An inflammatory reaction of some degree usually is to be found at the margin of the invading and less often at that of the noninvasive growths. The reacting cells are chiefly of the small round cell kind (figs. 7 and 19).

10. Nemilof, A.: Arch. f. mikr. Anat. **72**:575, 1908.

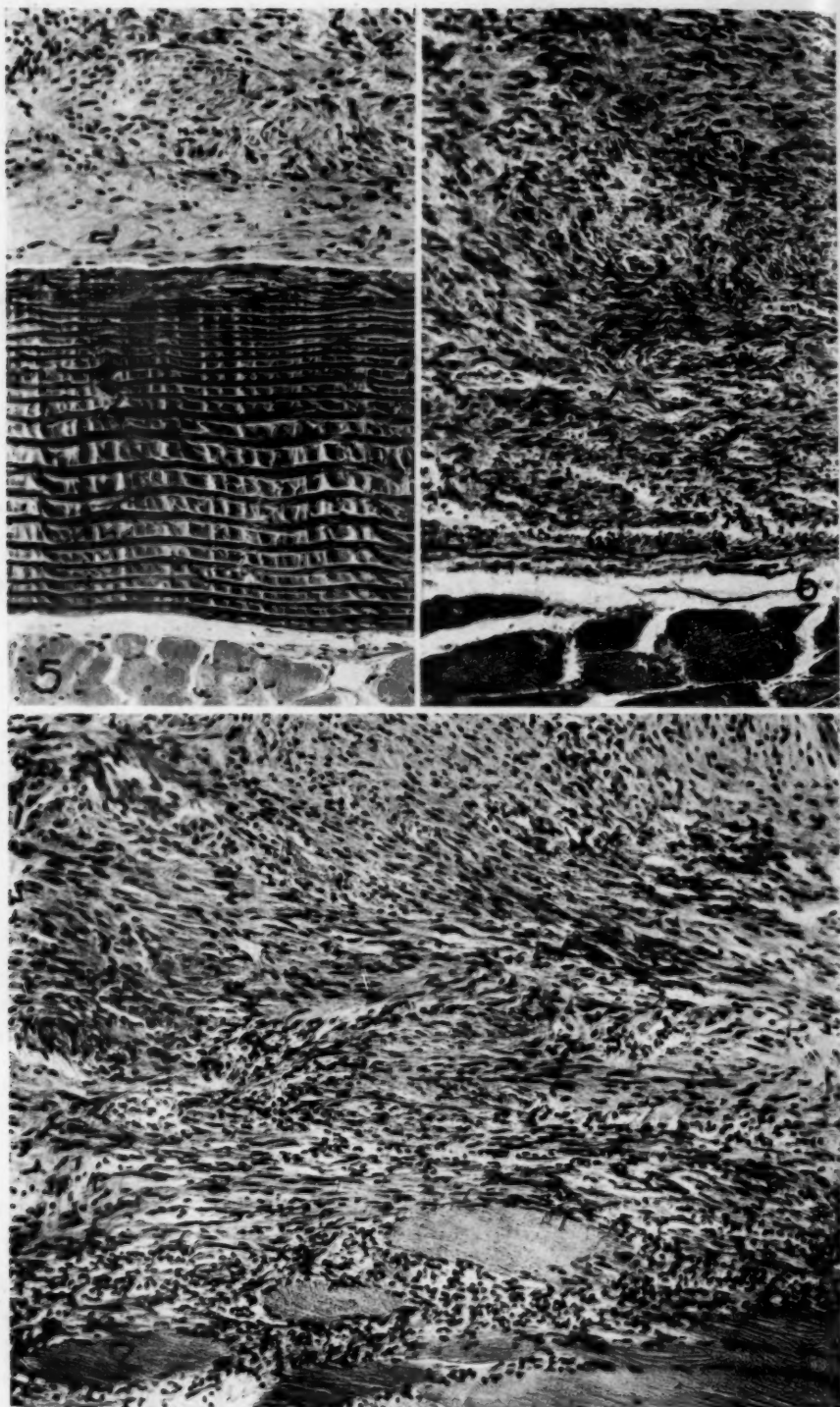
11. Drs. P. Masson, of the University of Montreal, Chandler Foot, of Cornell University Medical College, A. P. Stout, of the College of Physicians and Surgeons of Columbia University, and B. Alpers, of Jefferson Medical College, examined a number of my sections and offered helpful advice.

EXPLANATION OF FIGURES 1 TO 4

Fig. 1 (tumor 7).—A solitary round tumor arising in the corium of a gray snapper. The growth extends from the lateral line to the dorsal fin at the level of the seventh to eleventh dorsal spines. It measures 24 mm. in the greatest diameter and projects 11 mm. above the skin. The surface of the tumor is smooth, the scales having been destroyed by the growth. (Total length of fish 24 cm.)

Fig. 2 (tumor 75).—Multiple tumors in a dog snapper. Three tumors are located in the corium on the left side. Two of the growths touch the lateral line. The largest measures 49 by 40 by 20 mm. and is coarsely nodular. (Total length of fish 49 cm.)

Figs. 3 and 4.—Anatomic distribution of the tumors. It is seen that the majority lie along and dorsal to the lateral line (shown as a broken line), particularly in an area just back of the head. There are relatively few tumors on the lower half of the body and but a single tumor (which is not shown) on the ventral surface. The tumors are drawn to approximate scale.



Figures 5 to 7
(See legends on opposite page)

In sum, the great majority of the tumors, though they lack a distinct capsule, are sharply bounded. The occasional tumors which are less sharply separated from the neighboring tissues have at most acquired limited power of invasion.

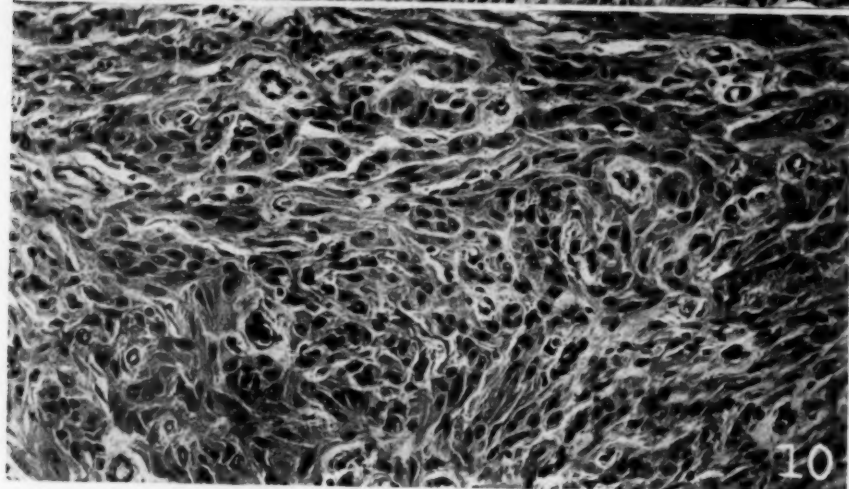
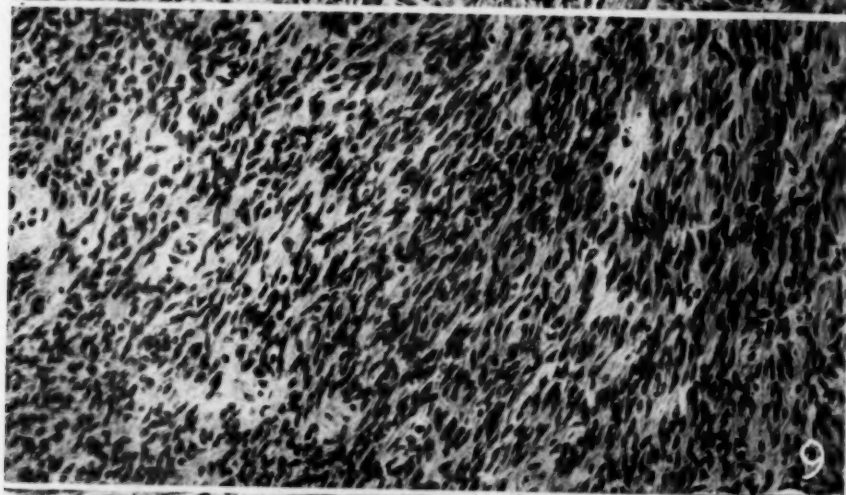
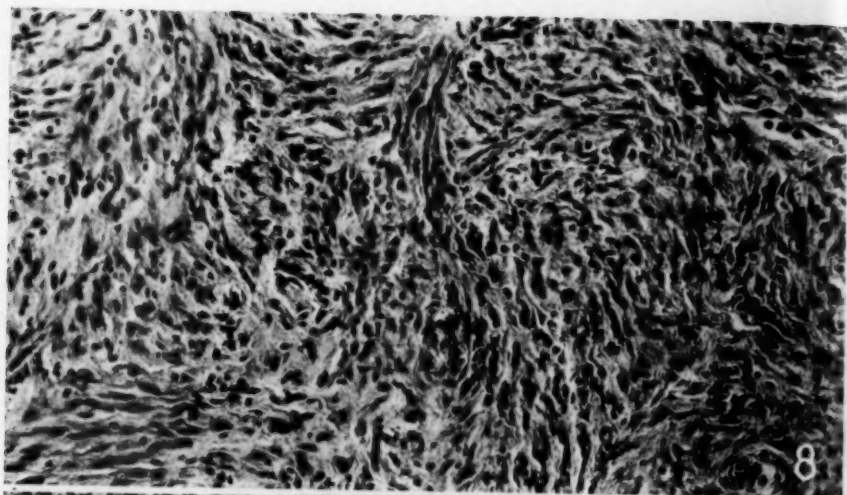
All of the tumors have three principal components: elongated cells which in shape resemble fibroblasts; intercellular fibrils, both of the collagenous and the argentophilic variety, and nonfibrillar material of a hyaline or mucinoid kind. The relative proportions of these components and their arrangement vary widely in the different tumors and even in different parts of the same tumor. Two main patterns, however, may be recognized. One type is compactly fibrocellular, the cells and fibers often being alined in such a manner as to form interlacing bands, whorls, parallel rows or, more rarely, complex structures resembling nerve end-organs. The other type has a loose and edematous pattern of growth and no particular arrangement of the components (fig. 14). These two types correspond closely to the Antoni A and B types of human nerve sheath tumors. In the present series both types usually coexist, sometimes one, sometimes the other predominating. Thus richly cellular islands may lie within loose reticulated regions (fig. 12), or the converse may obtain. There are some tumors, however, which are purely fibrocellular, and others which are composed of loose reticulated tissue exclusively. When two or more neoplasms occur in the same animal, the structure of the several tumors usually differs considerably.

EXPLANATION OF FIGURES 5 TO 7

Fig. 5 (tumor 67).—This photograph shows the relation of the tumor to the subjacent tissue. It is seen that the base of the tumor is sharply limited by the stratum compactum, the inner portion of the corium, which in the photograph appears as a broad ribbon composed of dense bundles of fibroconnective tissue in parallel arrangement. Between the stratum compactum and the muscles lies the subcutaneous fatty-areolar tissue, which in the area shown is scanty. This is an example of a tumor which lies entirely within the outer, loose portion of the corium. $\times 180$. (From a flat oval tumor, measuring 10 by 4 mm., located just below the lateral line at the level of the first dorsal ray.)

Fig. 6 (tumor 7).—The tumor has completely destroyed the stratum compactum but has not invaded the subjacent subcutaneous tissue. It is sharply delimited and not invasive, although it has no capsule. This is an example of a tumor which occupies the entire corium. $\times 180$. (From the tumor shown in fig. 1.)

Fig. 7 (tumor 47).—The tumor has destroyed the corium, invaded the subcutaneous tissue and infiltrated and destroyed the superficial parts of the muscle. Note the well marked fasciculated arrangement of the tumor, and the small round cell reaction at the periphery. $\times 200$. This is an example of a tumor having limited powers of invasion. (From a hemispherical tumor, 19 by 19 by 8 mm., which was situated across the lateral line at the level of the second to fourth dorsal rays. Grossly the tumor was not limited by the corium but extended for several millimeters into the muscle. It had definite connections with nerves.)



Figures 8 to 10
(See legends on opposite page)

The main variants met in the cellular, Antoni A type are illustrated in figures 8 to 10. Nearly all of the tumors contain interlacing fasciculi (fig. 8) or broader ribbons of cells in more or less parallel alinement which, like schools of fish, appear to stream in the same direction (fig. 9). The individual cells are poorly outlined, narrow and compressed, with thin, elongated or shorter oat-shaped nuclei and scanty cytoplasm. It is particularly in tumors having this structure that intercellular fibers may be conspicuous. With connective tissue stains, delicate to coarse collagenous strands may be seen, or, less often, broader nonfibrillar hyaline bands. In close association with the collagenous fibers are numerous argentophilic fibers. In fasciculi they may be traced for a considerable distance as black straight or wavy threads which pass between the tumor cells (fig. 16).

Areas composed of much larger cells with abundant cytoplasm are frequently encountered, especially as islands within loose reticulated type B tissue (fig. 10). These cells are frequently gathered in syncytial rows or in more irregular multinucleated masses. The nuclei are oval and have one or two small nucleoli and scattered chromatin granules; mitotic figures are rare.

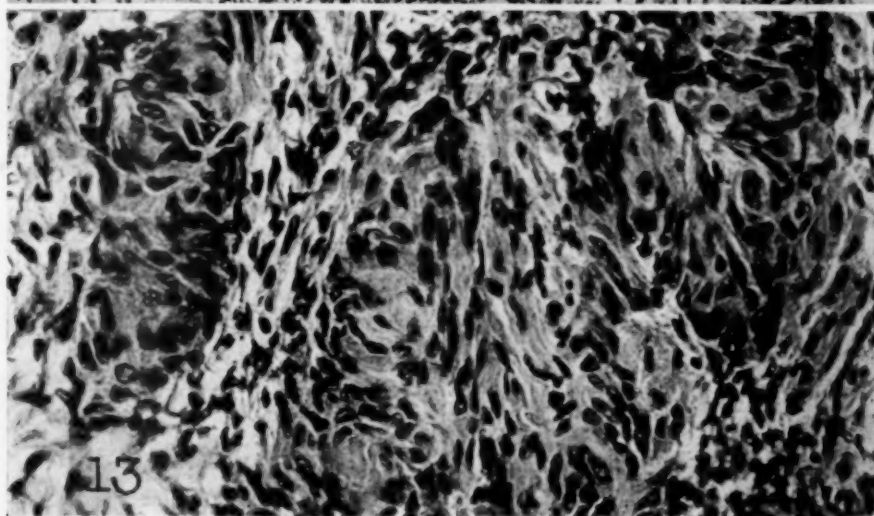
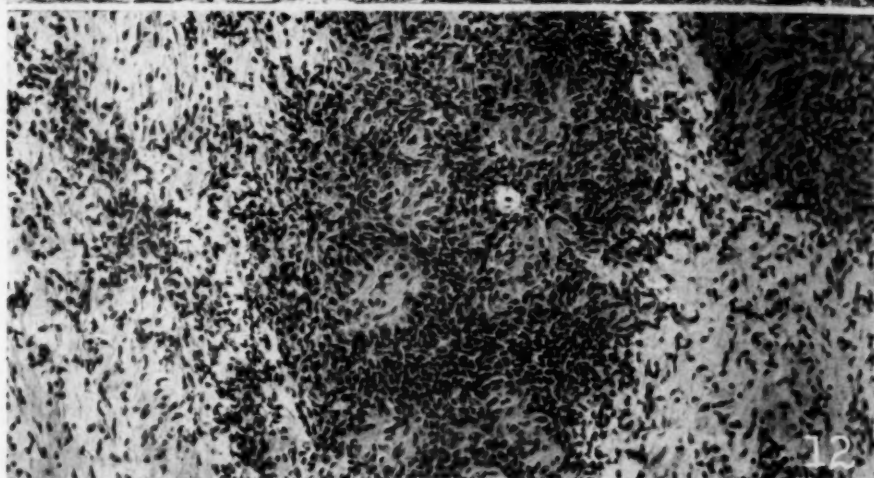
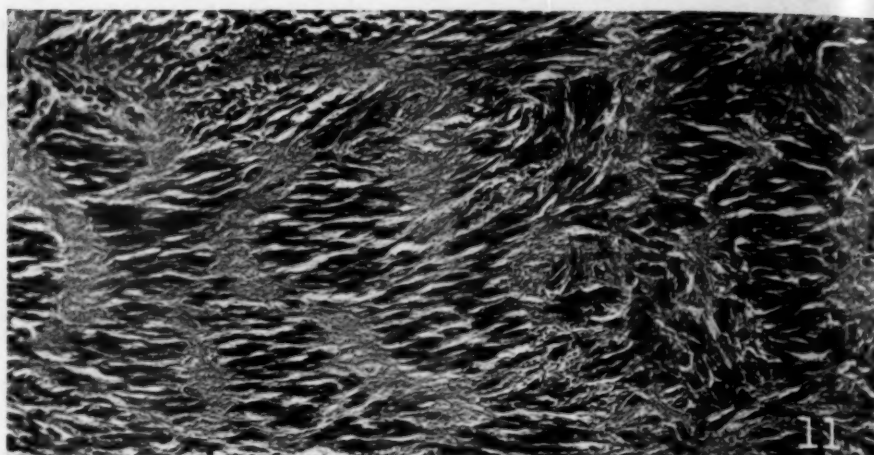
Yet another, and the most distinctive, feature of these tumors is shown in figure 11. Conspicuous elongated nuclei, enveloped by scanty and indistinct cytoplasm, lie side by side in rows; connecting these nuclear rows or palisades ("parades," "stockades") are closely packed

EXPLANATION OF FIGURES 8 TO 10

Fig. 8 (tumor 23).—A dense fibrocellular area of a tumor. The cells together with the abundant fibers form tightly woven bundles which interlace in various directions. $\times 280$. (From the smaller of two tumors; it had a flat, oval shape, measured 11 by 7 by 4 mm, and was located somewhat posterior to the left pectoral fin. The larger tumor had in most parts a loose areolar structure; elsewhere laminated corpuscles were present, of the type shown in fig. 19.)

Fig. 9 (tumor 29).—A broad bundle of tumor cells is shown in which nearly all of the cells are oriented in the same direction and hence are parallel to one another. There is relatively little intercellular substance. The nuclei are oat shaped; the cytoplasm is poorly outlined and scanty. $\times 280$. Sections from other areas of the tumor have the appearance shown in figures 12 and 13. (From a coarsely nodular tumor on the snout which involved the nares; it measured 17 by 12 by 7 mm.)

Fig. 10 (tumor 34).—The tumor cells are large, with abundant cytoplasm and oval nuclei; in many areas they form a multinucleated syncytium. They are arranged in irregular rows, with a tendency toward the formation of small whorls. The photograph is from a cellular island of a tumor which in nearby areas had a loose reticulated texture and elsewhere was densely fasciculated. $\times 280$. (The tumor occurred near the right pectoral fin in a large dog snapper; it measured 19 by 16 by 6 mm.)



Figures 11 to 13
(See legends on opposite page)

fibrils, all running in the same direction. Such palisade formations are of inconstant occurrence in human nerve sheath tumors but when present are regarded as almost pathognomonic. In the fish tumors, palisading is met in approximately one third, occurring sometimes in many areas of the individual tumor, sometimes in but few.

The evolution of the palisades has been studied especially by Masson,¹¹ who traces them to schwannian elements and points out that these formations may vary greatly in complexity; even organoid structures resembling nerve end organs may result from progressive differentiation. Such more complex formations are encountered in a few of the fish tumors. In figure 12 are shown rounded structures with marginal nuclei and a densely fibrillar interior. In figure 13 (which is from the same tumor) are seen similar but more elongated formations which resemble the corpuscles of Meissner. They are constructed of piles of cells which lie side by side and transversely to the long axis of a fibrillar core; the entire formation is well delimited. Organoid formations of this kind in tumors of fish are of interest, as Meissner's corpuscles have not been found in vertebrates other than mammals.¹² But it is well known that sensory end organs vary greatly in form and that the named varieties, such as the corpuscles of Meissner, represent but arbitrary types which themselves are widely variable.

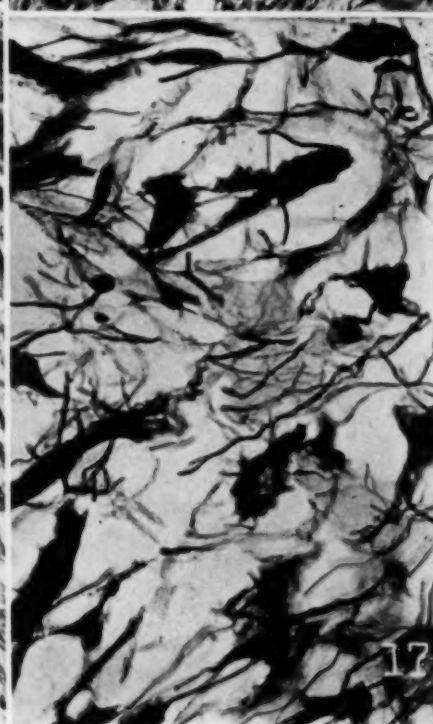
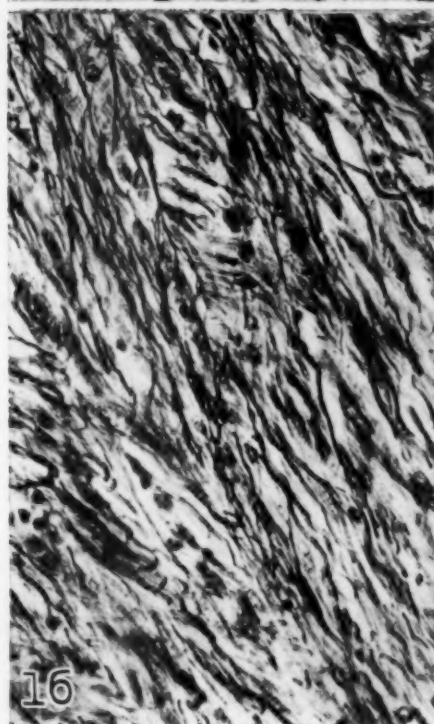
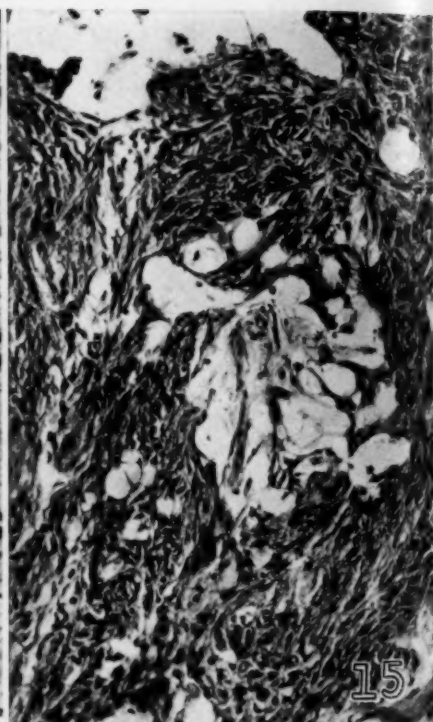
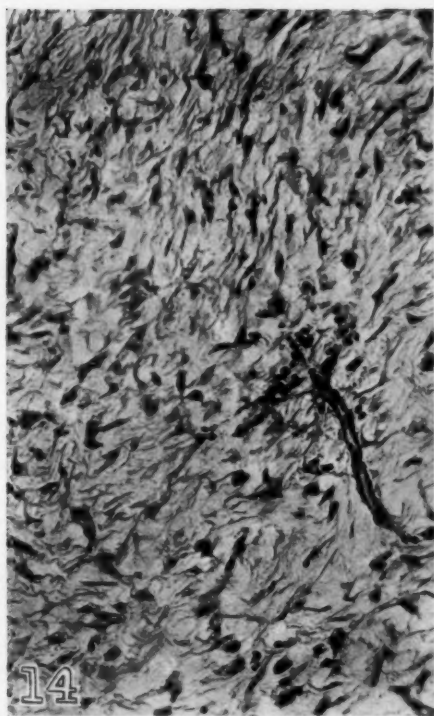
12. Boeke, J.: *Nerve Endings, Motor and Sensory*, in Penfield, W.: *Cytology and Cellular Pathology of the Nervous System*, New York, Paul B. Hoeber, Inc., 1932, sect. 6.

EXPLANATION OF FIGURES 11 TO 13

Fig. 11 (tumor 30).—The photograph shows the palisading of the nuclei which is considered pathognomonic for neurilemmoma. The elongated nuclei with their poorly outlined, scanty surrounding cytoplasm are arranged side by side, in rows; the nuclear rows are connected by masses of delicate fibrils, the prolongations of which pass between the cells. $\times 280$. (From a large tumor, measuring 41 by 22 by 12 mm., on the right side of a dog snapper. The tumor lies across the lateral line at the level of the fourth soft dorsal ray. A much smaller tumor, 6 mm. in its greatest diameter, was located on the left side of the head somewhat posterior to the eye.)

Fig. 12 (tumor 29).—In the central part of the photograph is shown a cellular nodule in which some of the component cells form whorls. The interior of these rounded structures is more or less fibrillar, with the cells arranged at the periphery (see also fig. 13). On either side of the nodule is loose reticulated tissue in which cells are widely separated (Antoni's B type of tissue). Note the abrupt transition from closely packed cells to poorly cellular loose reticulated tissue. In still other parts the tumor forms fasciculi as shown in figure 9. $\times 122$.

Fig. 13 (tumor 29).—The tumor cells form structures which closely resemble certain nerve end organs, such as the Meissner corpuscles of mammals. $\times 500$.



Figures 14 to 17
(See legends on opposite page)

Up to this point I have been concerned with an account of the compact fibrocellular type of neoplastic tissue. A strikingly different arrangement is seen in the loose, Antoni B type (fig. 14). Here the cells are widely separated; they lie in various planes and exhibit no attempt at orientation. Cytoplasmic processes of individual cells are often seen to anastomose with those of neighboring cells. Collagen strands are usually thin; the argentophilic fibers are short and curly (fig. 17). The substance which separates the cells and fibers is probably a very watery material, for it disappears almost completely in the preparation of the sections. Only occasionally traces remain, which sometimes stain faintly with acid dyes, sometimes with basic dyes. There is no gradation between neoplastic tissue of types A and B. As shown in figure 12, the transition from compact fibrocellular to loose, poorly cellular, reticulated areas is abrupt.

The cells in the reticulated areas show no evidence of degeneration. On the contrary, they are clearly outlined and appear to be entirely intact. These facts make it difficult to interpret the Antoni B type as a degenerative phenomenon, a "myxoid" degeneration or a "jellyfication" of type A tissue, as it is commonly regarded. Rather, it appears more likely that the two types of neoplastic tissue result from proliferation of two different types of cells. Murray and Stout,¹¹ to whom this hypothesis is credited, on the basis of their tissue culture experiments hold that nerve sheath tumors may take origin from two biologically different kinds of Schwann cells, derived from medullated

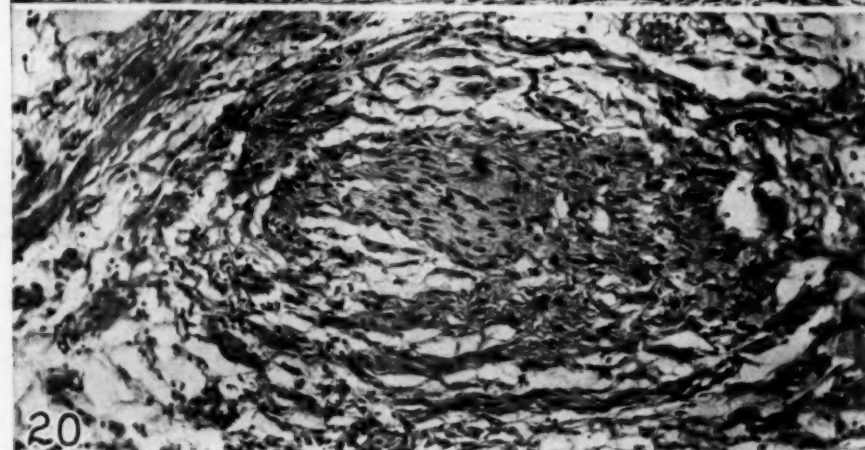
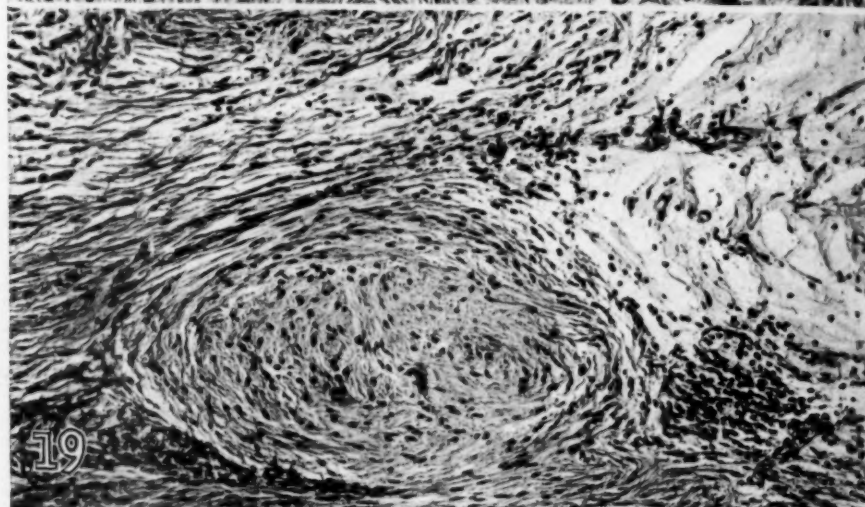
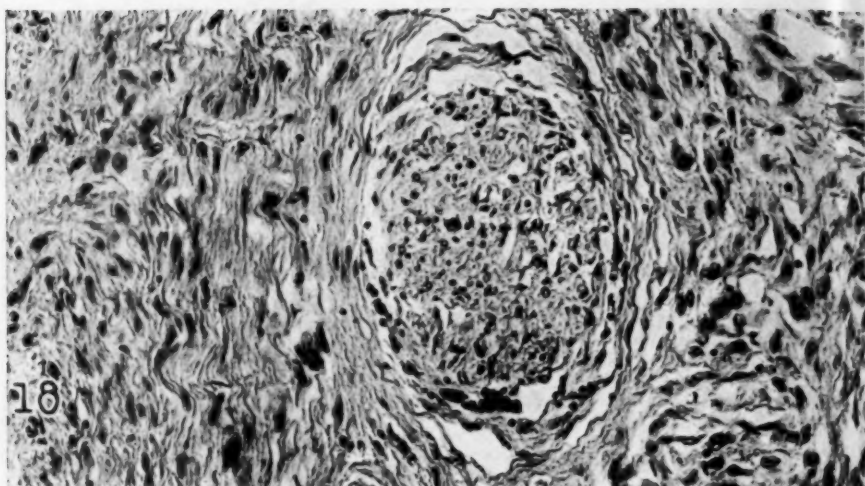
EXPLANATION OF FIGURES 14 TO 17

Fig. 14 (tumor 53).—The structural pattern of the tumor tissue is loose areolar or reticulated. The cells are widely separated either by fluid or by a very soft and watery intercellular substance (Antoni's B type of tumor tissue). $\times 280$. (From a tumor touching the right lateral line at the level of the second dorsal spine; it measured 10 by 8 by 6 mm.) Approximately one half of the tumor had a loose texture such as that shown, whereas the other half was densely cellular and fasciculated.)

Fig. 15 (tumor 30).—Areas of so-called microcystic degeneration. The cysts appear to have been formed from softening of hyaline intercellular substance (which is not shown in the photograph). The cysts have a distinct outline. In many parts of this tumor the cells have formed well marked palisades (fig. 11). $\times 180$.

Fig. 16 (tumor 1).—The section was stained with silver. Long straight or wavy argentophilic fibers are seen to run between the tumor cells. $\times 500$. (From a tumor below the left eye, measuring 15 by 9 by 5 mm.)

Fig. 17 (tumor 30).—The section was stained with silver. The area shown is from a region similar to that illustrated in figure 15. The argentophilic fibers are widely spaced and short, and some appear to have become fragmented. $\times 500$.



Figures 18 to 20
(See legends on opposite page)

and nonmedullated nerve fibers, respectively; or, alternatively, that the same type of cells may undergo different degrees of neoplastic proliferation.

While in the majority of these tumors of fish the cells appear to be separated by a thin watery material, in some tumors a more solid intercellular substance is present. In this material small cysts may appear, perhaps through enzymic digestion. These microcysts have a distinct outline (fig. 15); they represent the so-called microcystic degeneration described by Antoni and others in human nerve sheath tumors. To what extent the two processes, the separation of cells by fluid and the formation of cysts within an intercellular jelly, are related remains unknown. Associated with microcystic changes there have been described in human tumors hyaline changes of blood vessels (a hyaline type of arteriolar sclerosis). No example of this lesion has been observed in the fish tumors, the vessels of which are thin walled and not numerous.

The relation of nerves to tumors is inconstant. At the margin, and more especially at the base, of many tumors large, well preserved nerve trunks are often encountered; sometimes they are completely surrounded by neoplastic growths (fig. 18), but they are never found within the tumors. Evidently, these nerves are not an integral part of the tumors. They are of interest because encroaching tumors may bring about

EXPLANATION OF FIGURES 18 TO 20

Fig. 18 (tumor 34).—The oval structure in the center of the photograph is a nerve which was found near the base of the tumor shown in figure 10. The perineurial connective tissue sheaths are edematous and lie in intimate contact with the tumor cells. $\times 280$.

Fig. 19 (tumor 53).—A small oval-shaped laminated structure from near the base of the tumor shown in figure 14. The interior of the mass is eosin-staining granular debris. The lamellas are thin and blend with the adjacent reticulated tumor tissue. Note the small round cell reaction. $\times 180$.

Fig. 20 (tumor 12).—This laminated structure is one of several nodules lying within loose reticulated tumor tissue (Antoni's B type of tumor tissue). The lamellas at the periphery are collagenous bands in which lie thin, elongated nuclei. The central mass consists of a bundle of delicate fibrils and cells with more numerous, shorter and thicker oval nuclei than those in the peripheral lamellas. All of the cells within this bundle are oriented in the same direction and hence are parallel to one another. The appearance and arrangement of this are very similar to other cell bundles scattered throughout the tumor. $\times 280$. (From a tumor on the tail just below the posterior dorsal fin; the tumor, spherical in shape, 12 mm. in diameter, projected for a distance of 5 mm. above the surface. The base was poorly outlined. On microscopic examination two tumors were found, one external to the stratum compactum, another beneath it; the latter probably took its origin from deeper subcutaneous nerves.)

changes in nerves which perhaps explain the formation of the peculiar concentrically laminated nodules often encountered in human as well as in fish tumors. One phase of such formation is shown in figure 19. Here the nerve fibers have been completely replaced by a fibrous and poorly cellular mass, enveloped by the split lamellas of the outer nerve sheath. Another and more important phase is shown in figure 20. The lamellas are more edematous than those shown in the preceding photograph; the nerve fibers are replaced by a proliferating mass of cells, which are extending through the sheath. It is not improbable that formations of this kind in already established tumors represent repetitions of the initial stages in the evolution of these neoplasms.

SIZE, SEX AND CONDITION OF THE TUMOR-BEARING FISH

All of the fish in this series were well grown and mature; no tumors were found in small, i. e., young fish. The gray snappers ranged from 25 to 35 cm. in length; the dog snappers, from 45 to 65 cm. None of the fish showed evidence of malnutrition or external disease. Complete postmortem examination of the internal organs (but not of the central nervous system) was performed on the majority of the fish, and no tumors or other noteworthy changes in the viscera were disclosed. No predominance of either sex (determined from external examination of gonads) was noted in the tumor-bearing fish.

INCIDENCE; GEOGRAPHIC AND ZOOLOGIC DISTRIBUTION

Gray snappers tend to gather in schools which frequent the neighborhood of docks, wrecks, large coral heads and similar places affording shelter. The clear and shallow waters of the Tortugas permit minute inspection of the fish (with a glass bottom bucket such as is customarily used for viewing marine life), so that it is possible to note tumors or other surface lesions. By this means the majority of the tumors in this series were discovered.¹³ It is not possible to estimate closely the incidence of tumors in snappers, but inspection of many schools of fish and statements by fishermen of experience indicate that as many as 0.5 to 1 per cent of gray snappers may be affected, at least in the waters about the Tortugas. It is certain that these tumors are very common, for within the relatively short collecting period of approximately sixty days all of the 76 specimens of tumor-bearing fish were obtained. Most of these came from the Tortugas; some were taken near Key West,

13. Mr. Phil Saunders, for many years one of the collectors of the Tortugas Laboratory, made this study possible by his skill in discovering and obtaining tumor-bearing fish.

where many of the commercial fishermen were familiar with what they called "cancer fish." To what extent the neoplastic disease is distributed elsewhere has not as yet been investigated.

Information as to the zoologic distribution of these tumors is more precise: They were found only in members of the snapper family. Search among other species of fish and particularly among those frequenting the same localities as snappers led to the discovery of tumors, but not tumors of the kind found in snappers. No information is as yet available as to the factors which cause this family of fish to be so commonly afflicted with neoplasms of the nerve sheath.

TRANSPLANTATION EXPERIMENTS

Preliminary transmission experiments were inconclusive. In one series, bits from a tumor of a gray snapper were inoculated with a small Bashford needle beneath the integument of 10 other gray snappers. In other series, bits from two different tumors were implanted into the anterior chamber or the vitreous of the eye, in 30 snappers. The fish were kept in large life boxes, which proved inadequate to maintain them in good condition, all but a few dying before sufficient time had elapsed to permit the drawing of conclusions. The most promising results were obtained with fish inoculated intraocularly, a few of which lived for as long as eight to ten weeks. In them remnants of the tumors remained, though the results were obscured by infections. These experiments must be regarded as merely exploratory. Since snappers have been successfully kept for long periods in marine aquariums of various cities, the opportunity exists for investigating these tumors experimentally.

SUMMARY

Fish of the snapper family (Lutianidae) are commonly afflicted with tumors which resemble the nerve sheath tumors of man called, variously, neurinoma, neurolemmoma, schwannoma, perineurial fibroma or neurofibroma. Neoplasms of this kind have been observed in 76 fish of three species, the gray snapper (*Lutianus griseus*), the dog snapper (*Lutianus jocu*) and the schoolmaster (*Lutianus apodus*). Though many fish of other families were examined, no tumors of this variety were found.

The tumors generally occur along the course of the larger subcutaneous nerves, particularly those of the head and dorsal regions, as solitary or multiple, relatively large firm white masses. Like human neoplasms of the nerve sheath group, the tumors of fish are usually composed of two kinds of tissue: one compact and richly fibrocellular (corresponding to the Antoni A type); the other loose, reticulated and poorly cellular (Antoni B type). The component cells and intercellular

fibers of the tumors appear to be essentially the same, and arranged in similar patterns, in fish and man. Palisade formations of nuclei and fibrils, which when present are considered an almost pathognomonic feature of nerve sheath tumors of man, occur in approximately one third of the tumors of the present series. Unlike human tumors of this group, the fish tumors, though well circumscribed, are usually not encapsulated; a small proportion shows limited invasive power.

Nerve sheath tumors appear to be considerably more common in certain species of fish than in man. The frequency of occurrence of these tumors in readily available species, which can be maintained for long periods in marine aquariums, renders them favorable material for the study of an important group of neoplasms.

EFFECT OF CHORIONIC GONADOTROPIN ON THE SPREAD OF PARTICULATE SUBSTANCES IN THE SKIN OF RABBITS

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AND

PETER ZAPPASODI, B.S.

PHILADELPHIA

In a study¹ of the hereditary constitutional factors related to the resistance and the susceptibility to tuberculosis of certain rabbit families it was found that the spread of particulate substances in the skin of some of these families paralleled the spread of their tuberculosis. Thus the most susceptible family, F, was characterized by spread of intracutaneously injected india ink over a wide area and by rapid and widespread dissemination of tuberculosis from the portal of entry. The most resistant family, A, on the other hand, exhibited restricted spread of the dye and localization of tuberculosis to the portal of entry. Furthermore, it was found that the spread of this particulate substance as well as that of diffusible rabbit hemoglobin was a sex-limited character²; both substances spread over a wider area in females than in males. In view of this observation and in consideration of the well known and little understood increment in the incidence of human tuberculosis coincident with puberty it seemed desirable to determine whether the spread of dyes in the skin of rabbits is affected by sex hormones.

It was shown by Sprunt³ and confirmed by other observers that the administration of estrogens restricted the spread of india ink in the skin of rabbits. The corpus luteum hormones are known to be physiologically antagonistic to the estrogens, for not only do they inhibit follicular maturation and ovulation but, as shown by Allen and Meyer,⁴ 3 units of progesterone neutralizes 675 units of estrone (theelin). Therefore it seemed possible that chorionic gonadotropin, the luteinizing principle of human pregnancy urine, might have an enhancing effect on the spread of dyes in the skin of this animal.

From the Henry Phipps Institute, University of Pennsylvania.

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2. Lurie, M. B., and Zappasodi, P.: *Proc. Soc. Exper. Biol. & Med.* **42**:741, 1939.

3. Sprunt, D. H.: *Proc. Soc. Exper. Biol. & Med.* **42**:718, 1939. Sprunt, D. H., and McDerman, S.: *Endocrinology* **25**:308, 1939. Sprunt, D. H.: *J. Exper. Med.* **74**:81, 1941.

4. Allen, W. M., and Meyer, R. K.: *Anat. Rec.* **61**:427, 1935.

EXPERIMENTAL METHODS AND MATERIALS

Accordingly, the following experiment was undertaken. A group of 35 male and 37 female rabbits was selected. They belonged to the fifth, sixth and seventh inbred generations of the families A, C, D, F, G and H described in the study referred to.¹ The male and female rabbits were of approximately the same age and older than 3 months. They had been isolated in individual cages from the time of their weaning. The males were in one room and the females in another. This practically obviated sexual stimulation. Since ovulation, with its consequent cycles of physiologic sex hormone activities, is initiated only by sexual stimulation in the rabbit, these animals may be considered as physiologically at rest from the standpoint of the functioning of their sex hormones.

Because these rabbits were members of different families and since the extent of the spread of india ink varied greatly in these several families, it was deemed advisable to test the effect of chorionic gonadotropin on those rabbits in which the original spread had been previously determined rather than to estimate the spread of the dye in differing rabbits with and without the administration of chorionic gonadotropin.

The following procedure was carried out. Five-tenth cubic centimeter of a 1:5 dilution of autoclaved india ink in saline solution was injected intracutaneously into the caudal half of the right flank of each rabbit. The hair had been removed on the previous day with electric clippers. There was no inflammation of the depilated skin at the time of the injection of the dye. In the cephalic half of the same flank was injected 0.3 cc. of homologous rabbit hemoglobin, prepared according to the method described by Madinaveitia.⁵ The injection of india ink was made with a tuberculin syringe constructed on the principle described by Vondrak.⁶ Near the thumb rest of the plunger was fastened a metal collar. To this was attached a strip of metal as long as the plunger. The free end of this strip was bent at a right angle and moved along the calibrations on the outside of the barrel of the syringe. This insured the delivery into the skin of the exact amount of the deeply black ink suspension. The hemoglobin could be accurately administered by means of an ordinary syringe. Five and twenty-four hours, respectively, after the injection, the spread of each dye was determined as follows: The limiting edge of the stained skin was outlined with ink. A sheet of thin transparent cellophane was snugly and smoothly applied to the uniformly stretched skin. The outline of the stained area was then traced on the cellophane. The exact area of spread was then determined by a planimeter from the cellophane tracing. The readings represented the original areas of spread of the two dyes in these rabbits.

Four to five days after this original injection of dye, each rabbit received a single intravenous injection of 0.1 mg. of a highly purified extract in salt solution of the gonadotropic substance in human pregnancy urine, prepared by Gurin, Bachman and Wilson.⁷ This glycoprotein was given to us by Dr. Gurin, of the department of physiologic chemistry of the University of Pennsylvania. One microgram per kilogram of this material injected intravenously causes ovulation in mature postpartum rabbits. Forty-eight hours after the intravenous injection of 0.1 mg. of the gonadotropin into resting adult rabbits, corpora haemorrhagica appear, the walls of which are formed by cells in various stages of transformation, with numerous mitoses, and abundant fully developed corpora lutea cells. Some

5. Madinaveitia, J.: *J. Biochem.* **32**:1806, 1938.

6. Vondrak, J.: *Science* **92**:410, 1940.

7. Gurin, S.; Bachman, C., and Wilson, D. W.: *J. Biol. Chem.* **128**:525, 1939; **133**:467 and 477, 1940.

of the corpora haemorrhagica have been transformed into fresh corpora lutea. The effect of the chorionic gonadotropin on the spread of dyes in the skin of these rabbits was determined forty-eight hours after the administration of the gonadotropin by injecting intracutaneously into the left flank of the same rabbits the same amounts of ink and hemoglobin and in the same relative areas, respectively, as had been used four to five days previously. The spread of these dyes was determined by planimeter in exactly the same manner as was described in the foregoing paragraph.

RESULTS

Spread of India Ink.—In chart 1 and its legend are presented the data as well as the graphic curves of the distribution in 37 female rabbits of given areas of spread of india ink in the skin one day after its injection as observed before and after treatment with the luteinizing gonadotropin.

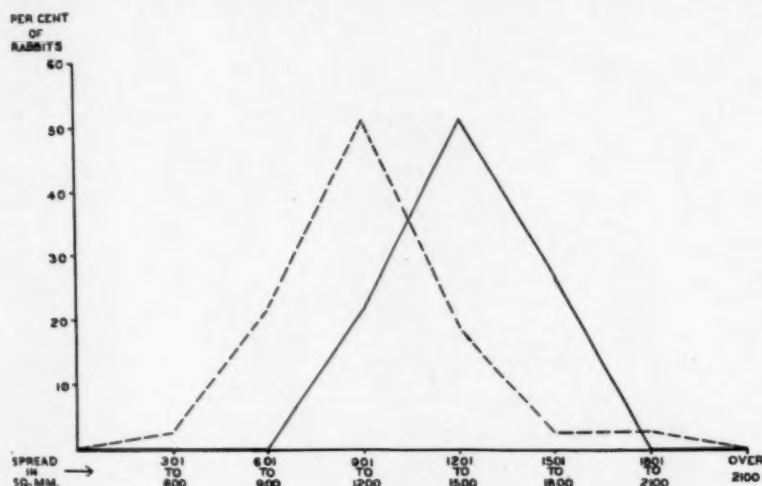


Chart 1.—Effect of chorionic gonadotropin on the spread of india ink in the skin of female rabbits twenty-four hours after the injection of the dye. The broken line represents the curve of spread before and the solid line that after the administration of gonadotropin. The data may be tabulated as follows:

Spread in Sq. Mm.	Rabbits Showing Given Spread			
	Before Treatment		After Treatment	
	Number	Per Cent	Number	Per Cent
301 to 600.....	1	2.7	0	0
601 to 900.....	8	21.6	0	0
901 to 1,200.....	19	51.3	8	21.6
1,201 to 1,500.....	7	18.9	19	51.3
1,501 to 1,800.....	1	2.7	10	27.0
1,801 to 2,100.....	1	2.7	0	0

The mean spread before treatment was 1,064 sq. mm. with a standard deviation of the mean of ± 43 . The mean spread of the ink in the same rabbits and in the same relative regions of skin after the administration of 0.1 mg. of this highly potent substance was 1,360 sq. mm. with a standard deviation of the mean of ± 29 . While the difference is only

one of 296 sq. mm., or an increment of 27.8 per cent, analysis reveals that the difference is of unquestionable statistical significance, for the critical ratio of this difference is 5.7. The significance of the difference can be readily appreciated by the fact that in more than 75 per cent of these rabbits before the gonadotropic treatment the spread of the dye was from 301 to 1,200 sq. mm., whereas after treatment the spread of the dye in 78 per cent of them was from 1,201 to 1,840 sq. mm. The regularity of the two curves further attests their reliability.

It is noteworthy that not only was the area over which the ink spread greater after the gonadotropic treatment in the females but the intensity of the black stain was greater than before the administration of the gonadotropin. In other words, not only did the ink particles penetrate

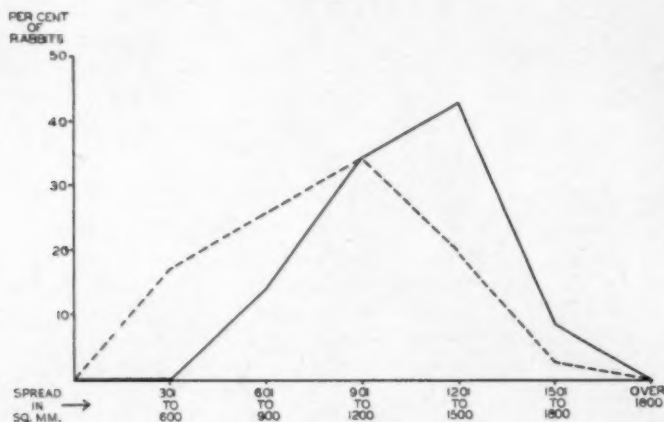


Chart 2.—Effect of chorionic gonadotropin on the spread of india ink in the skin of male rabbits twenty-four hours after the injection of the dye. The broken line represents the curve of spread before and the solid line that after the administration of the gonadotropin. The data may be tabulated as follows:

Spread in Sq. Mm.	Rabbits Showing Given Spread			
	Before Treatment		After Treatment	
	Number	Per Cent	Number	Per Cent
301 to 600.....	0	17.1	0	0
601 to 900.....	9	25.7	5	14.0
901 to 1,200.....	12	34.2	12	34.2
1,201 to 1,500.....	7	20.0	15	42.9
1,501 to 1,800.....	1	2.9	3	8.6
Over 1,800.....	0	0	0	0

a greater distance from the site of injection but a greater number of them left the site of deposition of the dye.

A similar though less marked effect was noted in males treated with this extract of the gonadotropic substance in pregnancy urine. The data as well as the graphic curves are presented in chart 2 and its legend. The mean spread of the india ink one day after the injection of the dye in the males before the gonadotropic treatment was 965 sq. mm. with

a standard deviation of the mean of ± 49 . After the administration of the gonadotropin the mean spread of this dye was 1,188 sq. mm. with a standard deviation of the mean of ± 39 . The average increment in spread following the administration of the gonadotropin was only 223 sq. mm. in the males, or 23.1 per cent. However, analysis shows that the difference was statistically significant, for the critical ratio of the difference was 3.5. Furthermore, examination of the data shows that in 22.9 per cent of the males the area of spread of the dye before treatment was from 1,201 to 1,800 sq. mm., whereas after the gonadotropic treatment the ink spread over this area in 51.5 per cent of them. It is noteworthy that in the females both before and after the administration of the gonadotropin the dye spread over a wider area than it did in the

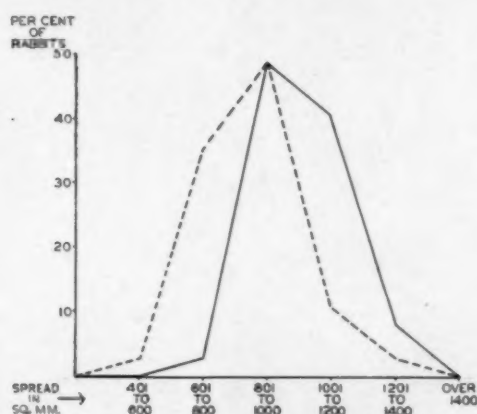


Chart 3.—Effect of chorionic gonadotropin on the spread of india ink in the skin of female rabbits five hours after the injection of the dye. The broken line represents the curve of spread before and the solid line that after the administration of the gonadotropin. The data may be tabulated as follows:

Spread in Sq. Mm.	Rabbits Showing Given Spread			
	Before Treatment		After Treatment	
	Number	Per Cent	Number	Per Cent
401 to 600.....	1	2.7	0	0
601 to 800.....	13	35.1	1	2.7
801 to 1,000.....	18	48.6	18	48.6
1,001 to 1,200.....	4	10.8	15	40.5
1,201 to 1,400.....	1	2.7	3	8.1
Over 1,400.....	0	0	0	0

males. This confirms the original observations² of the sex-limited character of the spread of the dye.

If the spread of india ink is determined five hours after the injection of the ink, a similar though much less pronounced effect of the gonadotropin is obtained. The spread of this dye in the skin of female and male rabbits before and after the gonadotropin treatment as measured five hours after injection of the dye is depicted in charts 3 and 4,

respectively. The average spread of the ink in females before treatment was 852 sq. mm. with a standard deviation of the mean of 22.6. After the administration of the gonadotropin the spread averaged 1,009 sq. mm. \pm 22.8. The increment in spread following the gonadotropic treatment was only 157 sq. mm., or 18.4 per cent. This increment, though considerably less than at the end of twenty-four hours, is nevertheless definitely of statistical significance, for the critical ratio of this difference is 4.9. This difference can be readily seen from the fact that before treatment the ink in 13.5 per cent of the female rabbits had spread, five hours after its injection, over an area of from 1,001 to 1,400 sq. mm. and that after treatment the dye was spread over this area in 48.6 per cent of them.

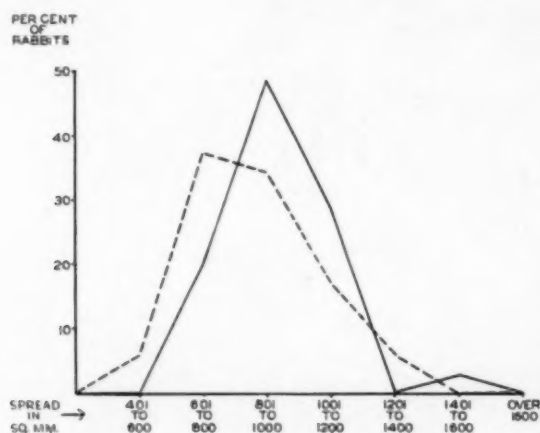


Chart 4.—Effect of chorionic gonadotropin on the spread of india ink in the skin of male rabbits five hours after the injection of the dye. The broken line represents the curve of spread before and the solid line that after the administration of gonadotropin. The data may be tabulated thus:

Spread in Sq. Mm.	Rabbits Showing Given Spread			
	Before Treatment		After Treatment	
	Number	Per Cent	Number	Per Cent
401 to 600.....	2	5.7	0	0
601 to 800.....	13	37.2	7	20.0
801 to 1,000.....	12	34.2	17	48.6
1,001 to 1,200.....	6	17.1	10	28.6
1,201 to 1,400.....	2	5.7	0	0
1,401 to 1,600.....	0	0	1	2.7
Over 1,600.....	0	0	0	0

In males the effect of the gonadotropin on the spread of india ink as measured five hours after its injection (chart 4) is much less pronounced; the increment in spread following treatment is only 8.5 per cent of the original spread. Furthermore, this difference is of no statistical significance, for its critical ratio is only 1.7.

Thus it is clearly seen that the administration of chorionic gonadotropin is followed by a greater dissemination of india ink in the skin of

both male and female rabbits, but the increment in females is more pronounced. This enhancement of spread is apparent both five and twenty-four hours after the injection of the dye, following the gonadotropic treatment. But the increment is more pronounced if the spread is determined twenty-four hours than if determined five hours after the injection of the dye.

Spread of Hemoglobin.—It was found in a previous study that the spread of homologous hemoglobin in the skin of rabbits is often indistinct twenty-four hours after its injection. Five hours after the intracutaneous introduction of this diffusible substance the margins of the hemoglobin-stained skin are very sharp. Hence the five hour spread of this dye is considered first.

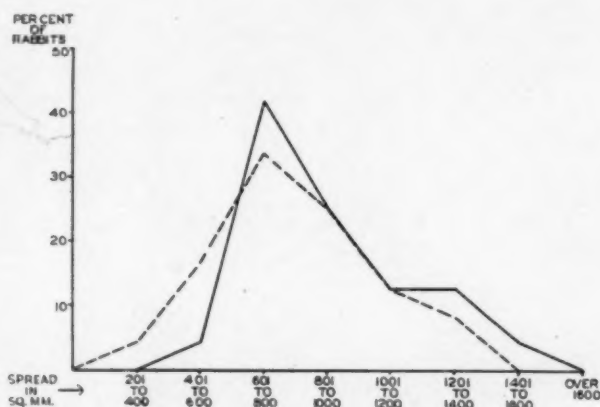


Chart 5.—Effect of chorionic gonadotropin on the spread of hemoglobin in the skin of female rabbits five hours after the injection of the dye. The broken line represents the curve of spread before and the solid line that after the administration of the gonadotropin. The data may be tabulated thus:

Spread in Sq. Mm.	Rabbits Showing Given Spread			
	Before Treatment		After Treatment	
	Number	Per Cent	Number	Per Cent
201 to 400.....	1	4.1	0	0
401 to 600.....	4	16.6	1	4.1
601 to 800.....	8	33.4	10	41.7
801 to 1,000.....	6	25.1	6	25.1
1,001 to 1,200.....	3	12.5	3	12.5
1,201 to 1,400.....	2	8.3	3	12.5
1,401 to 1,600.....	0	0	1	4.1
Over 1,600.....	0	0	0	0

The spread of diffusible hemoglobin in the skin of female and male rabbits as measured five hours after injection of the dye before and after treatment with the gonadotropin showed a tendency in the same direction as was found with particulate ink, namely, an increment of the spread after treatment. These data and their corresponding curves are presented in charts 5 and 6 with their legends, respectively. Thus the original

average spread of the hemoglobin in the skin of 24 female rabbits was 801 sq. mm. After administration of the gonadotropin this spread was 911 sq. mm. However, the difference is not statistically significant, for its critical ratio is only 1.6. Similarly the average original spread of hemoglobin in the skin of 35 male rabbits five hours after its injection was 637 sq. mm.; after the gonadotropic treatment the spread averaged 723 sq. mm. However, the difference is again only of limited statistical significance, for its critical ratio is only 2.3.

When the spread of the dye was determined twenty-four hours after the injection of the hemoglobin there was a statistically reliable increment of 19.2 per cent in females after the gonadotropic treatment as compared with the original twenty-four hour spread in these rabbits before the

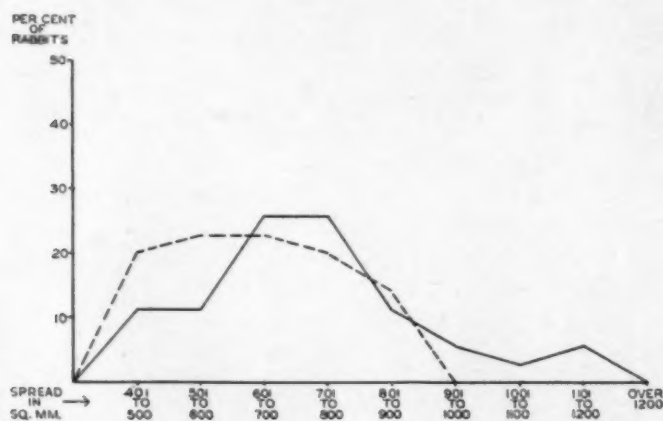


Chart 6.—Effect of chorionic gonadotropin on the spread of hemoglobin in the skin of male rabbits five hours after the injection of the dye. The broken line represents the curve of spread before and the solid line that after the administration of the gonadotropin. The data may be tabulated thus:

Spread in Sq. Mm.	Rabbits Showing Given Spread			
	Before Treatment		After Treatment	
	Number	Per Cent	Number	Per Cent
401 to 500.....	7	20.0	4	11.4
501 to 600.....	8	22.8	4	11.4
601 to 700.....	8	22.8	9	25.7
701 to 800.....	7	20.0	9	25.7
801 to 900.....	5	14.3	4	11.4
901 to 1,000.....	0	0	2	5.7
1,001 to 1,100.....	0	0	1	2.9
1,101 to 1,200.....	0	0	2	5.7
Over 1,200.....	0	0	0	0

gonadotropic treatment. In males the increment, while present, was less and of no statistical significance.

Thus it is clearly seen that both particulate and diffusible substances tend to spread in the skin of both males and females more widely after

the administration of the gonadotropin than before. Furthermore, twenty-four hours after the injection of these dyes the enhancing effect of the gonadotropin is pronounced. It is much less in evidence five hours after the introduction of the dyes. This indicates that the increment in the spread of a dye in the skin of a rabbit following administration of chorionic gonadotropin is a function of time. It is only when the effects of the gonadotropin on the tissues continue for one day that a constant increment of the dissemination of the dye introduced therein results. Five hours after the injection of the dye the tendency, while present, has not yet attained its full development.

The permeation of india ink in the skin can be clearly discerned twenty-four hours after the intracutaneous administration of the ink. The spread of hemoglobin, however, is less accurately determined at this time, for the margins are often very vague and at times the entire area of hemoglobin-stained skin has faded in twenty-four hours, presumably on account of a more rapid metabolic breakdown of the pigment in some rabbits. It is for this reason that the results obtained in regard to the effect of the gonadotropin on the spread of diffusible hemoglobin in the skin of rabbits are less certain.

The validity of ascribing the observed significant increment in the spread of particulate matter to the gonadotropic treatment is contingent on the condition that the spread of this substance does not vary significantly from time to time in the skin of the same rabbit. That this is the case is demonstrated in chart 7.

Twenty-nine rabbits belonging to the third, fourth, fifth, sixth and seventh inbred generations of the families A, B, C, D, F and H had been selected for another study.¹ Of these 29 rabbits, all but 6 were males. The spread of india ink in their skin was determined as previously described except that the area of dissemination was estimated by multiplying the two longest diameters of the stained area at right angles to each other instead of by planimeter. It may be stated here that direct comparison of areas determined by both these methods shows a constant relation, so that while planimeter readings are actually more nearly correct, the estimate of the area of spread based on the diameters gives relatively similar results. These rabbits were then subjected to a series of intracutaneous injections of heat-killed tubercle bacilli. On the sixty-third day following the beginning of treatment each of these same rabbits was again given an intracutaneous injection of india ink, and its spread was again determined in the same manner. It was found, as shown in chart 7, that the average spread of the dye before sensitization was 674 sq. mm. with a standard deviation of the mean of ± 73 . After sensitization with heat-killed tubercle bacilli the spread averaged 607 sq. mm. with a standard deviation of ± 74 . It is evident that there was no

significant difference in the spread of india ink in the skin of these rabbits on these different occasions. Therefore the increment observed following the administration of the luteinizing gonadotropin must be ascribed to the effects of this treatment on the permeation of particulate matter in the skin of both male and female rabbits.

In order to determine the duration of the effect of the gonadotropin on the capacity of the skin to allow spread of the ink 21 female and 17 male rabbits were selected from those tested previously. These rabbits were chosen for their greatest increase in the spread of india ink after the gonadotropic treatment. Fifty-one and fifty-five days, respectively, after the injection of the gonadotropin into these rabbits they were again given an intracutaneous injection of 0.5 cc. of a 1:5 dilution of autoclaved india ink in saline solution. Twenty-four hours later the spread

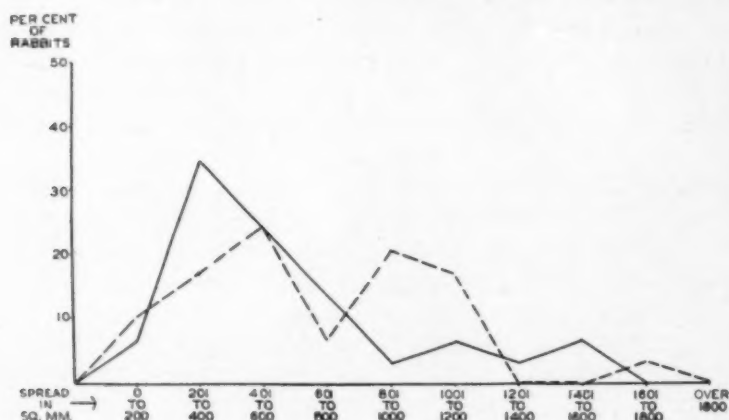


Chart 7.—Spread of india ink twenty-four hours after the injection of the ink into the skin of rabbits as observed before and after sensitization with heat-killed tubercle bacilli. The broken line represents the curve of spread before and the solid line that after sensitization. The data may be tabulated as shown here.

Spread in Sq. Mm.	Rabbits Showing Given Spread			
	Before Sensitization		After Sensitization	
	Number	Per Cent	Number	Per Cent
0 to 200.....	3	10.3	2	6.9
201 to 400.....	5	17.3	10	34.5
401 to 600.....	7	24.2	7	24.2
601 to 800.....	2	6.9	4	13.8
801 to 1,000.....	6	20.6	1	3.4
1,001 to 1,200.....	5	17.3	2	6.9
1,201 to 1,400.....	0	0	1	3.4
1,401 to 1,600.....	0	0	2	6.9
1,601 to 1,800.....	1	3.4	0	0
Over 1,800.....	0	0	0	0

of the dye in the skin of the male and female rabbits was again determined by planimeter as previously described. In chart 8 and its

legend are given the data and the corresponding curves of the distribution of given areas of spread of india ink in the skin two and fifty-one days, respectively, after the injection of the gonadotropin in female rabbits. In this chart are also included for comparison the data for the spread of the dye in these rabbits before the injection of the gonadotropin. It is clearly seen that not only has the enhancing effect of the gonadotropin on the spread of ink in the skin disappeared in fifty-one days but that the spread at this time is significantly less than the original permeation of the ink before injection of the gonadotropin. Thus the average spread of the ink in this group of rabbits before the treatment (dash line), was 976 sq. mm. with a standard deviation of the mean of ± 42 ; two days after administration of the gonadotropin (solid line), this spread averaged 1,368 sq. mm. with a standard deviation of the mean of ± 38 ; fifty-one

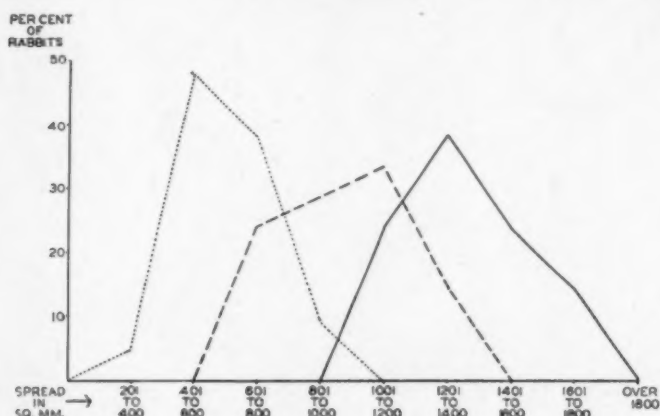


Chart 8.—Spread of india ink twenty-four hours after injection of the ink into the skin of female rabbits as observed before the administration of the gonadotropin and at two intervals after this treatment. The curve of spread before the gonadotropic treatment is represented by the line of dashes; that two days after the treatment, by the solid line, and that fifty-one days after the treatment, by the line of dots. The data may be tabulated in the following manner:

Spread in Sq. Mm.	Rabbits Showing Given Spread		
	Before Treatment	2 Days After Treatment	51 Days After Treatment
201 to 400.....	0	0	1
401 to 600.....	0	0	10
601 to 800.....	5	0	8
801 to 1,000.....	6	0	2
1,001 to 1,200.....	7	5	0
1,201 to 1,400.....	3	8	0
1,401 to 1,600.....	0	5	0
1,601 to 1,800.....	0	3	0
Over 1,800.....	0	0	0

days after administration of the gonadotropin (dotted line), the ink spread over an average area of only 614 sq. mm. with a standard deviation

of the mean \pm 26. These differences are all of high statistical significance. The critical ratio between the second and the third curve is 16 while that between the first and the third is 7.4. An examination of the curves clearly shows that the distribution of the spread of the dye on these three occasions was characteristic for each occasion. The median spread before administration of the gonadotropin was 980 sq. mm., which is very close to the average spread of 976 sq. mm. The median spread of the ink two days after this treatment was 1,350 sq. mm., while the average was 1,368 sq. mm. Finally the median spread on the fifty-first day after treatment was 600 sq. mm., compared with the average of 614 sq. mm. All these observations attest the statistical reliability of the observations.

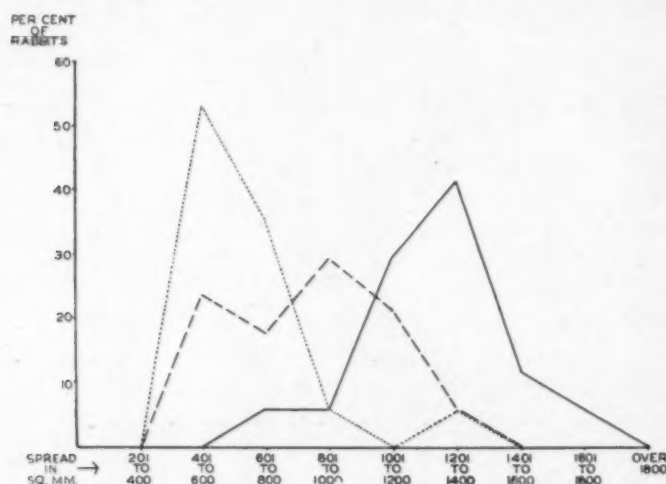


Chart 9.—Spread of india ink twenty-four hours after the injection of the ink into the skin of male rabbits as observed before the administration of gonadotropin and at two intervals after its administration. The curve of spread before the treatment is represented by the line of dashes; that two days after the treatment, by the solid line, and that fifty-five days after the treatment, by the line of dots. The data may be tabulated as follows:

Spread in Sq. Mm.	Rabbits Showing Given Spread		
	Before Treatment	2 Days After Treatment	55 Days After Treatment
201 to 400.....	0	0	0
401 to 600.....	4	0	9
601 to 800.....	3	1	6
801 to 1,000.....	5	1	1
1,001 to 1,200.....	4	5	0
1,201 to 1,400.....	1	7	1
1,401 to 1,600.....	0	2	0
1,601 to 1,800.....	0	1	0
Over 1,800.....	0	0	0

Essentially similar results were obtained in males. They are depicted in chart 9. In these, too, not only had the enhancing effect on the spread

of the dye in the skin disappeared fifty-five days after the administration of the gonadotropin, but, as was found in the females, the permeation of the ink in the skin was less than the original dissemination of the particulate matter before any gonadotropic treatment had been given. Thus before treatment the spread of the ink in these males (dash line) averaged 835 sq. mm. with a standard deviation of the mean of ± 56 . The median was 840 sq. mm. Two days after treatment (solid line) this permeation averaged 1,244 sq. mm. ± 55 with a median of 1,240 sq. mm., while fifty-five days after treatment (dotted line) this averaged 636 sq. mm. ± 50 , with a median of 590 sq. mm. The critical ratio between the first and the second curve is 5.2 and that between the second and the third is 8.1, but between the first and the third the ratio is only 2.6 or of relatively slight statistical significance.

COMMENT

Since Duran Reynals' ⁸ original discovery of the enhancing effect of testicular extract on the spread of various substances, including bacteria in the tissues of animals, bacterial products ⁹ and "leukotaxine" ¹⁰ of inflammatory exudates have been added to the list of spreading agents. In this group of substances may now be included the luteinizing gonadotropin of human pregnancy urine. A single intravenous injection of 0.1 mg. of this substance given to rabbits weighing 2 to 3 Kg. results forty-eight hours later in a statistically significant increment of approximately 20 to 30 per cent in the spread of india ink in the skin of both males and females as compared with the spread of this dye in the same rabbits before the gonadotropic treatment. This represents an activity of approximately 1 part in 25,000,000 if the observed effect is attributed to the direct action of the gonadotropin on the tissues. This, however, seems unlikely. The luteinizing extract used produces corpora lutea in the female ovary with the consequent release of progesterone in the blood. In the male the same extract stimulates the interstitial cells of the testis with elaboration of the male sex hormone, testosterone. That the urinary gonadotropin causes the formation of testosterone is demonstrated by the descent of the testis in cryptorchism following its administration.¹¹ It would appear more likely that the effect observed is traceable to the hormones progesterone and testosterone which this gonadotropin causes to appear.

It is of interest to note in this relation that the enhancement of spread following the gonadotropic treatment is observable twenty-four hours

8. Duran Reynals, F.: *J. Exper. Med.* **50**:327, 1929; **61**:617, 1935.

9. Duran Reynals, F.: *J. Exper. Med.* **58**:161, 1933.

10. Menkin, V.: *J. Exper. Med.* **67**:129, 145 and 153, 1938.

11. Thompson, W. O., and Heckel, N. J.: *J. A. M. A.* **112**:397, 1939.

after the injection of the dye but is less pronounced at the end of five hours. This suggests that the effect observed is cumulative and subject to the lapse of time rather than to an already achieved altered state of the tissues at the time of the injection of the test substances. This would be understandable if the tissues were subjected to a continuous action of a spreading agent released in the body, such as the progesterone of the corpora lutea, which originates through the action of the injected luteinizing gonadotropin of pregnancy urine.

The enhancing effect of this gonadotropin is more pronounced in the female than in the male. It has been generally accepted that the luteinizing agent in pregnancy urine is comparable in its effect on the gonads to the corresponding gonadotropic principle naturally elaborated by the anterior lobe of the pituitary. In fact, until recently it was designated as the anterior pituitary-like substance. This suggests that the observed greater natural spread of particulate and diffusible substances in the skin of untreated female as compared with that of untreated male rabbits may be accounted for by the differing effect of the gonadotropic principle of the pituitary on the male and the female, just as the luteinizing gonadotropin of human pregnancy urine has a greater enhancing effect on the spread of india ink in the female as compared with the male. This, however, is only a possibility and remains for further investigation to ascertain.

That the spreading effect of the gonadotropin derived from the anterior lobe of the pituitary plays a role in the great increment in the incidence of tuberculosis observed in man and particularly in young women coincident with the onset of puberty would seem to be possible on the basis of these observations. It has been shown by Walker and Hoffman¹² that the introduction of the spreading agent from testicular extracts at the site of inoculation enhances and accelerates the tuberculous process. Furthermore, as shown in the study of constitutional factors related to tuberculosis,¹ resistance to the disease is to a great extent a function of the localization of the infection. In resistant rabbits the infection tends to be restricted to the portal of entry. In susceptible animals it is permitted to spread widely in the tissues. Again, there is a correlation, though incomplete, between the natural capacity of the skin of animals to allow spread and their resistance to tuberculosis. For example, in one of the many tests of the relative resistance of the rabbits of the A and F families it was found that the average spread of india ink in the skin of 4 to 6 representatives of each of these families was 293 and 905 sq. mm., respectively, before infection. The average survival of these A rabbits was five hundred and thirty-nine days, while that of the F

12. Walker, T. D., and Hoffman, D. C.: *Am. J. Path.* 9:651, 1933.

rabbits was one hundred and forty-one days after an identical intracutaneous infection. Both observed differences are statistically significant. However, in some families of intermediate resistance and of heterogeneous constitution this correlation was absent. It appears, therefore, that one of the many factors related to resistance of rabbits to tuberculosis is the capacity of their tissues to allow the spread of particulate matter. Is it possible that with the onset of puberty there is a release of gonadotropic hormones from the pituitary, which, like the luteinizing factor of human pregnancy urine, enhance the capacity to allow spread of particulate matter and hence also of tubercle bacilli in the tissues? That the enhancing effect is more pronounced in the female would be in accord with the greater mortality from tuberculosis that is observed among adolescent women as compared with that among young men. This, however, merely suggests a line of investigation which may be pursued.

That the sex hormones play a role in the spread of particulate substances in the skin has been shown by Sprunt, as noted. It is noteworthy that the estrogens studied by him restricted the spread of india ink, i. e., an effect opposite that of the luteinizing gonadotropin. This is understandable, for it is well known that the luteinizing hormone suppresses ovulation and the formation of estrogens.

It has been noted in this study that the enhancement of the spread of particulate matter in the skin of rabbits can no longer be demonstrated fifty-one and fifty-five days after the administration of the luteinizing gonadotropin. In fact, in females in particular, the spread of india ink fifty-one days after the gonadotropic treatment appears significantly less than that observed before the gonadotropin was given. The significance of this observation is at present uncertain.

SUMMARY

One-tenth milligram of a highly purified extract containing the luteinizing factor of human pregnancy urine when injected intravenously into rabbits significantly enhanced the spread of india ink in their skin. This enhancement of spread was more pronounced in the females than in the males. The effect was observed forty-eight hours after the injection of the gonadotropin. Fifty-one days after this treatment the enhancement of spread was no longer demonstrable. In fact, in females the spread of india ink at this time was significantly less than that observed before the administration of the gonadotropin. The increment in spread was pronounced twenty-four hours after the injection of the test dye. Five hours after the injection it was less in evidence. It is suggested that the effects observed are due to the stimulation of the ovary

and the testis by this gonadotropin with the release into the blood of progesterone and testosterone in females and males, respectively. The results are discussed from the standpoint of the role that the corresponding gonadotropic hormone of the anterior lobe of the pituitary may play in the greater natural spread of particulate and diffusible substances in the skin of females as compared with that of males and also from the standpoint of the possible role of this hormone in resistance to tuberculosis, particularly in relation to the increment in the mortality from this disease in young men and young women coincident with puberty.

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CHEMOTAXIS

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PHILADELPHIA

Chemotaxis, as usually thought of by the pathologist, is essentially a reaction of leukocytes to bacteria, and consists in the movement of leukocytes toward bacteria, resulting in phagocytosis. That this conception is too narrow is appreciated by a brief survey of directional responses in the kingdoms of animals and plants. Of course, just how many such responses should be included under the term "chemotaxis" depends on how the word is defined. The definition proposed here is that chemotaxis is a reaction in which the direction of locomotion in the cell or organism is determined by a substance in its environment.

So defined, chemotaxis is found to be exhibited by organisms from the highest mammals to bacteria.¹

Let us take an example from near the top of the scale, the bird dog. The bird dog when questing moves more or less in circles until he picks up the scent. Then he changes direction, i. e., reacts with chemotaxis, and moves toward the bird. His path, however, is not straight but diverges a little to one side, a little to the other, as if he were following a radio beam, until the scent leads him to the bird. The chemical substance which elicits this response, i. e., the scent of the bird, is suspended in the air, and the dog by his sense of smell is able to appreciate that the scent is stronger in one direction (toward the bird) than in the opposite direction (away from the bird); i. e., there is a concentration gradient of an attracting substance, and the dog is able to detect this gradient. There is a close analogy between this reaction and the response of leukocytes to bacteria.

Thus in higher animals chemotaxis is mediated by the sense of smell. The attracting substance may be carried by air, as in the attraction of the male by the female moth, or by water, as when a piece of well rotted meat is thrown into the bay and crabs converge on it from all directions, guided toward it by chemotaxis.

Among plants, ferns possess chemotaxis, as was shown by Pfeffer² in one of the first papers on this type of directional response. The attracting substance is malic acid, which is said to guide the sperm to

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1. *Tabulae biol.* 4:356 and 437, 1927.

2. Pfeffer, W.: *Untersuch. a. d. bot. Inst. zu Tübingen* 1:363, 1884.

the egg cell. In flowering plants, pollen tubes are guided by carbohydrates³ or proteins⁴ in their growth toward the ovum.

In the instances thus far given chemotaxis is positive; i. e., the organism or cell moves *toward* the source of attraction. But negative chemotaxis is known equally well. The negative reaction is manifested in general by protozoa.⁵ When these organisms reach a place where the concentration of some dissolved substance is too high or too low, they show what is known as the avoiding reaction: They stop, turn and start off in a new direction. The result of this reaction is that protozoa move away from deleterious substances, i. e., display negative chemotaxis.

From these few examples, chemotaxis is seen to be of distinct advantage. Positive chemotaxis guides toward food or the mate and aids in fertilization. Negative chemotaxis enables the organism to avoid environments that are unfavorable or dangerous. Leukocytes likewise are guided by chemotaxis toward their prey, or away from possibly deleterious substances, as if their reactions were survivals of those of free living organisms.

A word as to the use of the term "negative chemotaxis." In the older literature "negative chemotaxis" is frequently used ambiguously, meaning either that the organism shows no positive directional response or that it is repelled by the chemotactic substance and moves away from it. Only the repelling effect is now spoken of as negative chemotaxis. Thus negative chemotaxis is the opposite of positive chemotaxis, and means that the organism or cell is impelled to move away from a certain substance. When the organism shows no directional response, this lack of reaction is spoken of as indifferent chemotaxis or, more accurately, as absence of chemotaxis.

It is unusual to find both positive and negative chemotaxis in the same organism. The occurrence of both types of reaction in free living organisms has been described, so far as I know, only in the slime molds (myxomycetes or mycetozoa). These organisms form slimy masses of various colors—yellow, white, purple or black—usually on the barks of trees. They travel along the bark by ameboid motion. Slime molds are particularly useful material for the study of chemotaxis in the laboratory, not only because they display both positive and negative reactions but because they can be studied both as specimens visible to the naked eye, several centimeters in diameter, and also, when cut into minute pieces, as microscopic objects. The slime mold chiefly studied in this country,

3. Miyoshi, M.: *Flora* **78**:76, 1894.

4. Lidforss, B.: *Ztschr. f. Botanik* **1**:443, 1909.

5. Jennings, H. S.: *Behavior of the Lower Organisms*, New York, Columbia University Press, 1931.

Physarum polycephalum, is attracted by dextrose (but not by sucrose) and is repelled by adequate concentrations of acids and alkalis.⁶ Thus, as just stated, chemotaxis serves to guide the organism toward food and away from substances that are harmful.

CHEMOTAXIS IN LEUKOCYTES

Leukocytes, like slime molds, display both positive and negative chemotaxis, while to many substances they are indifferent. Positive chemotaxis is exemplified in figure 1, in which is shown the response of polymorphonuclear leukocytes of the rabbit to collodion particles. It is seen that during the sequence, occupying eight minutes, the cells moved closer to the collodion particles, some in nearly straight lines, others by more circuitous paths. The leukocytes showing the best responses recall the bird dog and the path he travels—a little to one side, a little to the other side, until the quarry is reached. Why collodion particles attract leukocytes is uncertain. Since the solubility of collodion in water is practically zero, it is unlikely that the attraction is due to a concentration gradient of collodion. Possibly the particles have absorbed some attracting substance from the water in which they are suspended. The question wants investigation.

Negative chemotaxis is illustrated in figure 2. Here the repelling substance is a suspension of silicate known commercially as Lloyd's reagent. The negative reaction of leukocytes is seen to be just as strong as was their positive reaction to collodion (fig. 1); they move in nearly straight lines away from the source of repulsion. In escaping from the circle of silicate, some of the leukocytes were obliged to pass between and close to two particles of silicate, as if the repelling substances were in higher concentration within the circle than at the periphery.

The reason for the repulsion of leukocytes by Lloyd's reagent is unknown. Similar though weaker repulsion is exerted by kaolin⁷ (which, like Lloyd's reagent, is made from impure aluminum silicate (fuller's earth) and by silicic acid. Repeated washing of these substances in redistilled water does not affect their repelling effect.

Kinds of Leukocytes Showing Chemotaxis.—In mammalian blood there are three kinds of leukocytes—polymorphonuclears, monocytes (which are related to wandering cells of the tissue or histiocytes) and lymphocytes. Of these, chemotaxis is displayed chiefly by polymorphonuclears, both neutrophils and eosinophils.⁸ No information is

6. Coman, D. R.: Arch. Path. **29**:220, 1940.

7. McCutcheon, M.; Coman, D. R., and Dixon, H. M.: Arch. Path. **27**:61, 1939.

8. Ingraham, E. S., and Wartman, W. B.: Arch. Path. **28**:318, 1939.

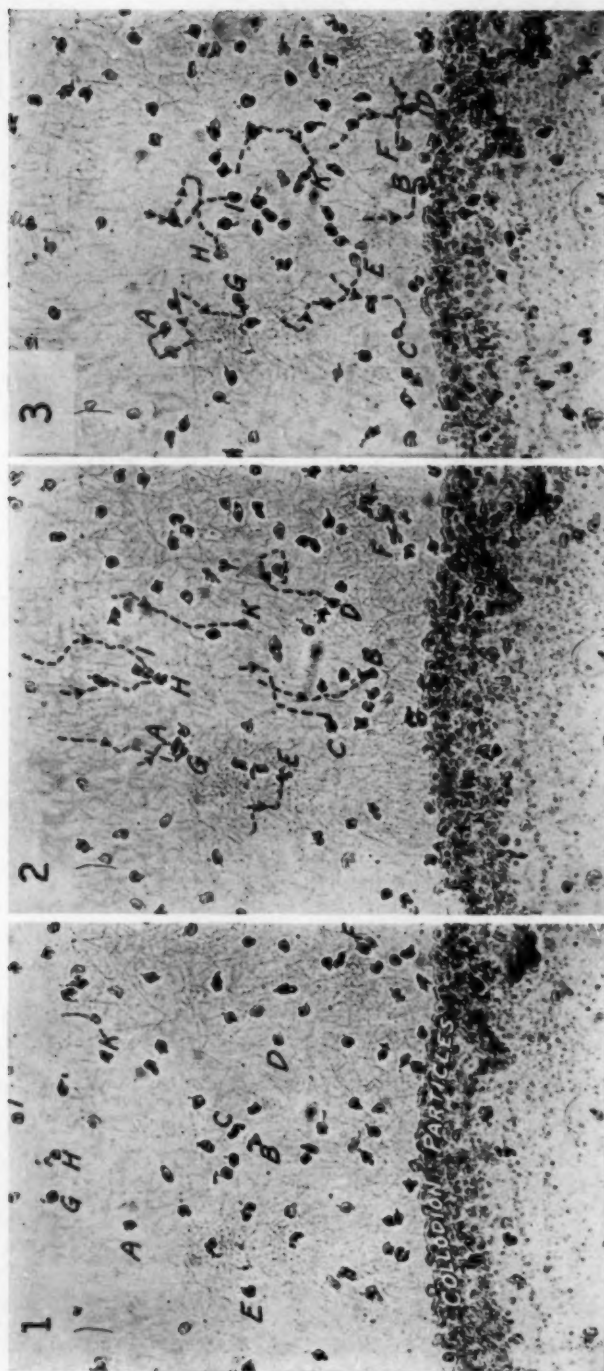


FIG. 1.—Positive chemotaxis of leukocytes. Polymorphonuclear leukocytes obtained from the peritoneal cavity of the rabbit, following injection of salt solution, were suspended in rabbit plasma and mounted between a slide and a cover slip (Coman, D. R.: *Am. J. M. Sc.* **196**:273, 1938). Collodion particles (Loeb, J.: *J. Gen. Physiol.* **5**:109, 1922) were used as the source of attraction.⁴² These particles are seen near the lower end of each photograph. The remainder of the field is occupied by polymorphonuclears in plasma. Photograph 1 shows approximately 90 leukocytes, of which 10, designated on the photographs by the letters A, B, C, etc., have been selected at random and their paths followed for eight minutes. This was done by projecting a motion picture film (made in collaboration with Drs. D. R. Coman and W. H. Lewis), the paths of the cells being recorded on paper, and these paths were then transferred to photographs 2 and 3. Photograph 2 shows the paths (in broken lines) of the 10 representative cells from 3:18 to 3:22 o'clock, and photograph 3 shows their further paths between 3:22 and 3:26 o'clock. It is seen that all 10 cells moved toward the collodion, some in nearly straight lines, others more indirectly. This is an example of positive chemotaxis.

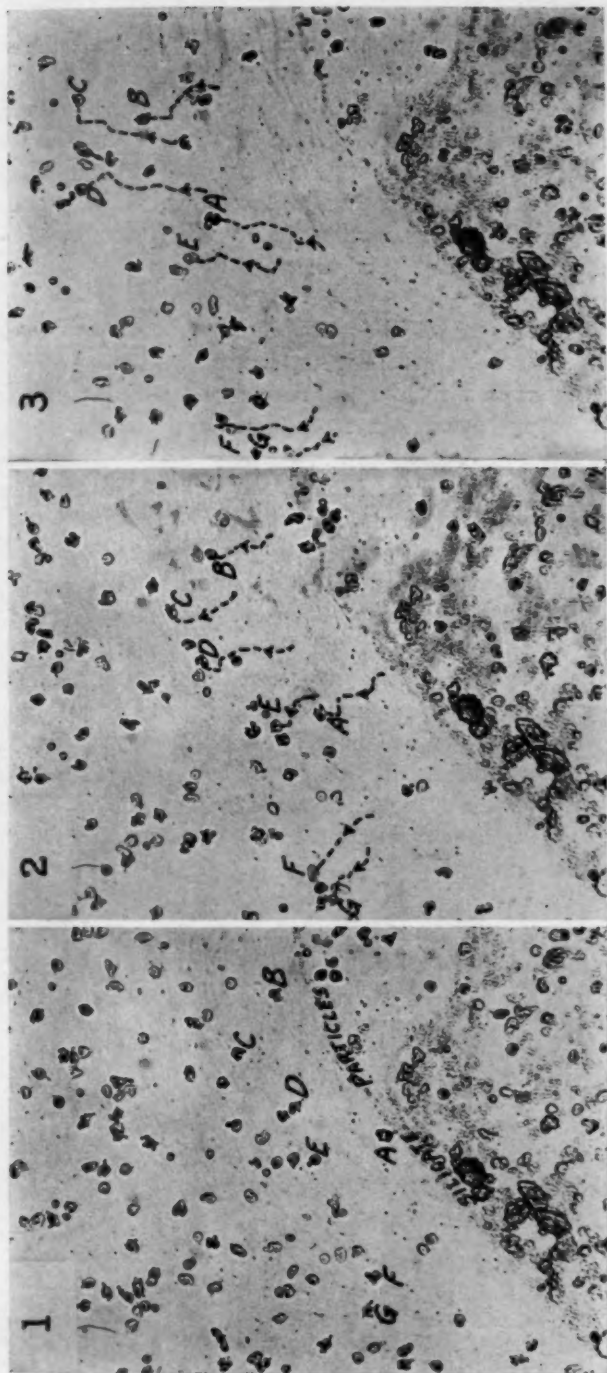


FIG. 2.—Negative chemotaxis. This experiment differs from that shown in figure 1 in that particles of aluminum silicate (Lloyd's reagent) 7 were used in place of collodion particles. The silicate particles are seen to occupy about one quarter of the field. Seven cells were selected at random and their paths followed for eight minutes, as explained for figure 1. All these leukocytes are seen to have moved away from the silicate, and to have done so in nearly straight lines, thus showing strong negative chemotaxis. In photograph 3 a clear zone is seen next to the silicate particles, indicating that nearly all the leukocytes in the field have moved away.

available in regard to basophils, nor can it be stated at what stage of maturation the granulocytes first display chemotaxis.

It seems appropriate that the leukocytes which chiefly display chemotaxis should be the polymorphonuclears. It is these cells that are first to emigrate from the blood vessels when bacteria have entered the tissues. These cells constitute the first line of defense. Quickly mobilized, they move rapidly toward the source of irritation in paths that are more or less straight and direct. Having reached the invaders they phagocytose and, if possible, digest them, and build a wall of cells around them that helps prevent their spread.

In this prompt response of the polymorphonuclears to irritants, chemotaxis is of great service. Were it not for this reaction, encounter of phagocyte and bacterium would be due merely to chance and therefore would require a longer time. But through their ability to react to bacterial products the leukocytes are guided directly toward the invaders and thus have opportunity to stop or limit infection before it can spread.

Monocytes and histiocytes have somewhat different functions. In most infections they are mobilized later. They constitute the second line of defense. They mop up after the polymorphonuclears, sweeping up surviving bacteria, dead tissue cells and debris. They move leisurely to and fro, gorge themselves, enlarge to become macrophages, divide, and then go forth to eat some more. Cells with these functions have no need to move directly toward invading organisms or other particles, and consequently it is not surprising that monocytes and histiocytes apparently do not display positive chemotaxis.⁹ They accomplish their ends by locomotion that is random. As to negative chemotaxis, there is evidence that these cells, like polymorphonuclears, are repelled by silicates, but this reaction is only weak.

In amphibians, however, pigmented macrophages have been observed to display positive chemotaxis¹⁰ and are described as moving directly toward starch granules and other substances *in vivo*. It is quite possible that mammalian monocytes, also, under other experimental conditions would show positive chemotaxis.

Lymphocytes, the third type of leukocyte, are so abundant in the blood and lymph that they presumably serve ends of great importance, but just what their functions are remains somewhat of a mystery.

Protection of the body against foreign proteins may be an important function of lymphocytes.¹¹ Since lymphocytes are capable of emigration from blood vessels¹² and apparently accumulate in regions of inflamma-

9. Coman, D. R.: *Arch. Path.* **30**:896, 1940.

10. Clark, E. R.; Clark, E. L., and Rex, R. O.: *Am. J. Anat.* **59**:123, 1936.

11. Rich, A. R.; Lewis, M. R., and Wintrobe, M. M.: *Bull. Johns Hopkins Hosp.* **65**:311, 1939.

12. Clark, E. R., and Clark, E. L.: *Am. J. Anat.* **46**:149, 1930.

tion, especially of the chronic sorts, it might be expected that they would exhibit chemotaxis. All present evidence, however, indicates that they do not.¹³ Though in in vitro experiments they move almost as rapidly as polymorphonuclears,¹⁴ their paths appear always to be at random. In the same microscopic fields in which the granulocytes are seen moving directly toward staphylococci or tubercle bacilli, lymphocytes travel either toward, away from or parallel to the clump of micro-organisms, in paths that appear to be determined only by chance. Caseous material from tuberculous lungs does not attract them.¹⁵ Nor, so far as known, are they repelled by any substance.

If, then, lymphocytes do not exhibit chemotaxis, how is it that they accumulate in regions of chronic inflammation? The answers suggested to this question are that they may be chemotactically attracted by substances not yet tested in vitro; that they may be trapped in inflamed areas, being free to wander in but prevented in some way from leaving the area; that they accumulate not so much through immigration as through multiplication in situ; that "small round cells" are not really lymphocytes at all but either cells of the fixed tissues or degenerated polymorphonuclears.¹⁶ Further investigation is certainly needed to clear up the many uncertainties that surround lymphocytes.

Substances That Excite Chemotaxis in Polymorphonuclear Leukocytes.—A surprisingly long list of micro-organisms and chemical substances have been tested for their chemotactic effect on leukocytes. The older literature has been admirably reviewed by Wells.¹⁶ Not all the results can be unreservedly accepted since some of the methods employed by earlier investigators appear to have been affected by serious errors.¹⁷

The really important chemotactic substances are derived from two sources, micro-organisms and damaged tissue cells. It is these substances almost exclusively that attract leukocytes in vivo, and to these the present paper will be largely confined.

Bacteria in general attract leukocytes,¹⁸ and in acute experiments in vitro, in which the intensity of the leukocytic reaction was measured, no differences were found in response to various bacteria.¹⁹

13. Dixon, H. M., and McCutcheon, M.: Arch. Path. **19**:679, 1935. Coman.⁹ Clark, Clark and Rex.¹⁰

14. McCutcheon, M.: Am. J. Physiol. **69**:279, 1924.

15. Dixon, H. M., and McCutcheon, M.: Am. J. Path. **11**:872, 1935.

16. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

17. McCutcheon, M.; Wartman, W. B., and Dixon, H. M.: Arch. Path. **17**:607, 1934.

18. Exceptions were noted by earlier investigators (Gabritchevsky, G.: Ann. Inst. Pasteur **4**:346, 1890).

19. McCutcheon, M., and Dixon, H. M.: Arch. Path. **21**:749, 1936.

In longer experiments, in which the tissue culture technic was used, pyogenic cocci were found to attract leukocytes from a greater distance than did some other bacteria, such as tubercle bacilli.²⁰ In vivo some bacteria attract leukocytes more than do others, though it seems likely that all bacteria attract when first inoculated. Pyogenic cocci in vivo attract more leukocytes and for a greater length of time than do most other micro-organisms. Tubercle bacilli in experimental infections attract polymorphonuclears during the first day or two, and again after about a week, when tissue cells begin to break down and liberate bacteria.²¹ Even typhoid bacilli are said to attract granulocytes when first injected.²²

Apparently, both living and dead bacteria excite chemotaxis. This statement must be qualified, however, by stating that some dead bacteria may always be present along with living bacteria, so that it might be true that bacteria attract only when they are dead.

The origin and the nature of the substances which attract leukocytes to bacteria are known only incompletely. It might even be asked whether the attracting substance does not come from damaged tissue cells instead of from bacteria, becoming attached to bacteria by adsorption. This is probably not generally true, as bacteria induce chemotaxis even when prevented from coming into contact with tissue.²³ That chemotactic substances are produced by bacteria themselves is true of at least some bacteria. Thus leukocytes are attracted by tubercle protein,²⁴ and by leukotaxine derived from *Staphylococcus aureus*.²⁵

There is, however, another way in which bacteria or other injurious agents cause accumulation of leukocytes. That is by causing attracting substances to be liberated from damaged tissues and cells. Burns and wounds of all sorts liberate tissue products that are chemotactic.²⁶ The chemical nature of products of damaged tissue has been intensively studied by Menkin,²⁷ who finds the active substance is a polypeptide, which he terms leukotaxine. The same substance, as stated above,²⁵ has been recovered by him from *Staph. aureus* and may prove to be present in other bacteria as well. Thus it may be that the substances of chief importance in chemotaxis, both those derived from bacteria and those from injured tissue cells, are split products of protein.

20. Meier, R.: *Ztschr. f. exper. Med.* **87**:283, 1933.

21. Woodruff, C. E.: *Am. J. Path.* **10**:739, 1934. Lurie, M. B.: *J. Exper. Med.* **60**:163, 1934.

22. Opie, E. L.: *Arch. Int. Med.* **5**:541, 1910.

23. Dixon, H. M., and McCutcheon, M.: *Proc. Soc. Exper. Biol. & Med.* **38**:378, 1938.

24. Wartman, W. B.: *Arch. Path.* **26**:694, 1938.

25. Menkin, V.: *Dynamics of Inflammation*, New York, The Macmillan Company, 1940, p. 59.

26. Moon, V. H.: *Arch. Path.* **20**:561, 1935.

27. Menkin, V.: *J. Exper. Med.* **67**:145, 1938.

That leukocytes are attracted by injured tissues has been known a long time. Damaged leukocytes,¹⁷ muscle²⁸ and especially skin²⁹ have recently been shown to be chemotactic. There is also evidence³⁰ that various carbohydrates attract leukocytes, though the significance of this fact for pathology is obscure.

WHAT DOES CHEMOTAXIS ACCOMPLISH?

The generally accepted view is that chemotaxis of leukocytes brings about their emigration from blood vessels in inflamed areas, directs their approach through the tissues to micro-organisms and helps in the movement of leukocytes from the sinuses of the bone marrow into blood vessels. Let us consider these three processes.

1. First, what is the evidence that chemotaxis is responsible for emigration? When the capillaries of the tadpole's tail are irritated—for example, mechanically—the capillary endothelium is seen to become less rigid and leukocytes adhere to it.¹⁰ This phenomenon is interpreted as increased stickiness of the endothelial cells. The leukocytes, also, appear to become more sticky.³¹ After adhering to the endothelium, some of the leukocytes begin to emigrate. Having passed through the wall of the capillary, they wander through the tissues without definite direction unless tissue has been damaged, in which case they move toward the damaged region.¹⁰

Their passage through the capillary wall may not be influenced by chemotaxis. Having stuck to the endothelium, leukocytes are stimulated to ameboid motion, as they are by contact with any solid or semisolid surface. If they must move, there is practically no place to go, except through the capillary wall. Their passage through the wall is presumably made easier by dilation and stretching of the wall and probably by softening of the endothelium.³² Thus it seems likely that emigration may occur even in the absence of chemotaxis, a view that is supported by observations of emigrating lymphocytes and monocytes,¹² cells which have not been found to exhibit positive chemotaxis.

But if there is a source of attraction in the outside tissues, the picture becomes different; the leukocytic reaction is much more intense. Thus, if a drop of croton oil is injected into the tadpole's tail, far more leukocytes adhere to the vascular endothelium, and great numbers emigrate.¹⁰ Chemotaxis appears greatly to increase emigration, or rather, to state the relation more accurately, conditions favoring chemo-

28. Grand, C. G., and Chambers, R.: *J. Cell. & Comp. Physiol.* **9**:165, 1936.

29. Silverman, D.: *Arch. Path.* **25**:40, 1938.

30. Chambers, R., and Grand, C. G.: *J. Cell. & Comp. Physiol.* **8**:1, 1936.

31. Baron, H., and Chambers, R.: *Am. J. Physiol.* **114**:700, 1936.

32. Clark, E. R., and Clark, E. L.: *Am. J. Anat.* **57**:385, 1935.

taxis also favor emigration. Whether the attracting substances arising in the perivascular tissues actually pass through the wall of the vessel is a matter of doubt. Older views that emigration is due to higher acidity in the tissues than in the blood,³³ or the more recently expressed view that emigration is brought about by differences in electric potential between the injured area and the blood,³⁴ lack confirmation.

Correlation between chemotaxis and emigration was shown in another series of experiments³⁵ in which saline solution was injected into the rabbit's peritoneal cavity. When the saline solution was prepared with doubly distilled water, emigration was scanty. Samples of saline solution prepared in this way were extracted with carbon, which was then washed and tested for its attraction of leukocytes *in vitro*. There was no attraction. If, however, the saline solution was made with distilled water prepared without special precautions, injection of this solution into the peritoneal cavity was followed by abundant exudation of leukocytes; correspondingly, in *in vitro* experiments saline solution prepared with ordinary distilled water and adsorbed on carbon was found to contain material that was chemotactic. Therefore, it is concluded that emigration of leukocytes may occur even in the apparent absence of attracting substances, but when these are present, emigration is intensified.

2. It is probably after leukocytes have emigrated that chemotaxis plays its important part in directing polymorphonuclears toward bacteria. The cells advance by ameboid movement and use as support fibrin, connective tissue fibers or other solid or semisolid structures. Leukocytes crawl but apparently do not swim. The paths traversed by leukocytes from blood vessels to the source of irritation may be nearly straight. Such a straight approach was observed after a drop of croton oil had been injected into the tadpole's tail¹⁰; in experiments *in vitro* the straightness of the path traversed by leukocytes has been used as a measure of the intensity of chemotaxis.¹³

As the result of such direct approach, leukocytes reach bacteria or other foreign material more rapidly and in greater numbers than if there were no chemotaxis. The chemotactic reaction leads the leukocytes into contact with the foreign particles. Without actual contact, phagocytosis is impossible. Thus, in the absence of chemotaxis, leukocytes may be seen to pass within a few microns of bacteria and yet not touch them.

Hence chemotaxis is an important part of the defense mechanism by which leukocytes combat infection. It is responsible for the speed and efficiency with which polymorphonuclears are mobilized about bacteria. The bacteria are then either phagocytosed or surrounded and walled off.

33. Feringa, K. J.: *Arch. f. d. ges. Physiol.* **203**:663, 1924.

34. Abramson, H. A.: *J. Exper. Med.* **46**:987, 1927; *J. Gen. Physiol.* **11**:743, 1928.

35. Coman, D. R.: *Am. J. Path.* **15**:597, 1939.

Negative chemotaxis of leukocytes was described in an earlier paragraph, as induced in vitro by silicates. Does negative chemotaxis occur in vivo? Certain substances, especially lactic acid,³⁶ formed in inflammatory foci have been reported to prevent accumulation of polymorphonuclears, but it is not clear whether this effect is due to negative chemotaxis, i. e., actual repulsion of leukocytes, or, more likely, to a toxic effect that inhibits locomotion. Obviously, if some poisonous substance prevents the cells from moving, they will be unable to accumulate. Such an effect should not be designated negative chemotaxis, since it has nothing to do with chemotaxis.

The same considerations apply to "negative chemotaxis" induced by bacteria. Thus it has been stated³⁷ that leukocytes react with positive chemotaxis to nonvirulent bacteria but with negative chemotaxis to virulent ones. But do such virulent organisms induce negative chemotaxis or, rather, inhibit locomotion, through such toxic substances as leukocidins? To distinguish between these two effects it is necessary to follow the paths of leukocytes with the microscope, to observe whether the cells actually move away from bacteria or whether they merely do not move. I⁷ have observed rabbit leukocytes near clumps of virulent streptococci; some of the cells seemed actually to be repelled, i. e., to exhibit negative chemotaxis, but the evidence for this was not entirely convincing.

Whether leukocytes show negative chemotaxis to bacteria is not at present certain. If they should do so during an infection, such a reaction would presumably be unfavorable to recovery. Finally, it may be remarked that it would be astonishing were cells to possess a function (negative chemotaxis) which they display only in vitro.

3. Whether the passage of leukocytes from the bone marrow into the blood stream is due to chemotaxis is actually a matter of conjecture, though such a mechanism is generally taken for granted. Somewhat opposed to this explanation are the observations of Menkin.³⁸ He found that leukocytosis is brought about not by thermostable leukotaxine but by thermolabile substances in the exudate which, unlike leukotaxine, do not excite the vascular response nor cause accumulation of leukocytes. Much the same considerations apply to immigration of leukocytes into blood vessels as to emigration. It may be that leukocytes in the bone marrow, having been stimulated to locomotion, enter the blood vessels because they must go somewhere.

In immunity, is chemotaxis increased? An affirmative answer might have been anticipated, because in immune states there is frequently

36. Menkin, V., and Warner, C. R.: *Am. J. Path.* **13**:25, 1937.

37. Petterson, A.: *Centralbl. f. Bakt. (Abt. 1)* **106**:294, 1926. Stevenson, J. W., and Reed, G. B.: *J. Bact.* **40**:239, 1940.

38. Menkin, V.: *Am. J. Path.* **16**:13, 1940.

marked increase in phagocytosis. Yet it was found that the leukocytes of rabbits immunized against certain streptococci show no greater chemotaxis toward those bacteria than do cells of rabbits that are not immunized. Nor does the serum of such immunized rabbits excite a greater chemotactic response toward those streptococci as compared with normal serum.³⁹ From present evidence, admittedly meager, chemotaxis is not affected by antibodies but is due to substances produced by microorganisms or by damaged tissues. Chemotaxis appears, unlike phagocytosis, not to be an acquired immune reaction. Rather it appears to be more primitive than specific immune reactions; it represents the survival of a primitive feeding reaction.

Neither through immune reactions nor otherwise has chemotaxis been experimentally increased. To bacteria, at least in vitro, leukocytes, if they react at all, react with maximal intensity. The reaction has a curious fixity of intensity, unlike phagocytosis, the intensity of which, under experimental procedures, varies widely. Consequently, no therapeutic procedure has been devised for increasing chemotaxis, though a possible beginning in this direction has been made by injecting chemotactic substances into tumors of rats, to induce leukocytes to accumulate.⁴⁰

Nor does any one know of diseases in which chemotaxis is much decreased. In some severe infections, it is true, leukocytes in inflamed areas are scanty, but this appears to be due more to loss of motility than to loss of chemotaxis. However, slight decrease in chemotaxis (but much greater decrease in motility) has been noted in leukocytes of acutely ill persons as compared with those of patients not acutely ill.⁴¹ Such decreased function would tend to retard the arrival of leukocytes in areas of infection, and thus to interfere with recovery.

MECHANISM OF CHEMOTAXIS

In discussing the mechanism of chemotaxis it should first be made clear that chemotaxis is a response superimposed on locomotion (e. g., in leukocytes, ameboid motion). Indeed, leukocytes move as fast when there is apparently nothing to attract them as when they are being attracted by bacteria.⁴² Thus chemotaxis is modified locomotion, modified as to direction. Consequently a theory of chemotaxis must take account of the mechanism of ameboid motion.

Ameboid motion, according to modern investigators, depends on continual changes in the colloidal state of protoplasm, through which

39. Coman, D. R.; McCutcheon, M., and DeCamp, P. T.: *Proc. Soc. Exper. Biol. & Med.* **41**:119, 1939.

40. Chambers, R., and Grand, C. G.: *Am. J. Cancer* **29**:111, 1937.

41. Mallery, O. T., Jr., and McCutcheon, M.: *Am. J. M. Sc.* **200**:394, 1940.

42. Dixon, H. M., and McCutcheon, M.: *Proc. Soc. Exper. Biol. & Med.* **34**:173, 1936.

jelled protoplasm becomes sol and vice versa. As the cytoplasm in the rear end of the cell becomes liquefied, it is pushed forward by contracting jelled cytoplasm, either because the gel is under tension⁴³ or because it contracts much as a muscle does.⁴⁴ Thus there is a continual flowing forward of liquid protoplasm from the posterior to the anterior end, where it constitutes a pseudopod. This pseudopod advances as the result of pressure from behind, though its advance must also be influenced by surface tension relations at the interfaces. As it advances, the sides of the pseudopod become changed from sol to gel, completing the reversible gel \rightleftharpoons sol transformation.

Thus the forward movement of the ameba, leukocyte or slime mold is active, not passive. It requires transformation within the cell of chemical energy into kinetic. Though ameboid motion may be imitated by means of models, such as a drop of mercury, the resemblance is only superficial. The living cell is not a drop of liquid, but a complex sol-gel system. Though the "ameboid" movement of the drop of mercury may be shown to be governed by surface tension, the mechanism of movement of the living cell appears to be more complex.

Chemotaxis, as already stated, is a *directional* reaction of the living cells superimposed on ameboid motion. As the bird dog is able to perceive that the scent is stronger when he moves toward the quarry, so the leukocyte is able to detect that the concentration of the attracting or repelling substance is stronger on the cell surface directed toward the quarry than on the opposite side.

Such a directional response may be imitated in models. A drop of mercury in a solution of nitric acid can be made to move toward a crystal of potassium dichromate,⁴⁵ and in a solution of potassium dichromate, can be made to move away from a drop of concentrated nitric acid,⁴⁶ these movements being regulated by surface forces. By analogy it has been stated¹⁶ that the directional responses of leukocytes are similarly regulated by surface forces. Against this explanation are the following objections: 1. The living cell is not a drop of liquid, like mercury, but a complex colloidal system and moves as the result of reversible gel-sol transformations. 2. Lymphocytes are exposed to the same external surface forces as polymorphonuclears and move by essentially the same mechanism,⁴⁷ yet lymphocytes apparently do not display chemotaxis though polymorphonuclears do so in the same microscopic field in which

43. Mast, S. O.: *Physiol. Zool.* **5**:1, 1932.

44. Lewis, W. H.: *Arch. f. exper. Zellforsch.* **23**:1, 1939.

45. Bernstein, J.: *Arch. f. Physiol.* **80**:628, 1900. Rhumbler, L., in Abderhalden, E.: *Handbuch der biologischen Arbeitsmethoden*, Berlin, Urban & Schwarzenberg, 1923, vol. 5, sect. 3, pt. 2, p. 219.

46. Coman, D. R.: Personal communication to the author.

47. Lewis, W. H.: *Bull. Johns Hopkins Hosp.* **55**:273, 1934.

the lymphocytes move at random.¹³ Hence it appears likely that chemotaxis is not due primarily or solely to tensions on the cell surface but to gel-sol transformations in the cell interior. Chemotaxis is excited by unequal concentrations of certain substances on different parts of the cell surface. The reaction may be pictured as the orientation of the sol-gel transformations, so that, e. g., in positive chemotaxis, gelation comes to be localized in the end of the cell nearest the source of attraction, while solation is localized in the opposite end. As the result of this orientation, solated protoplasm is squeezed toward the source of attraction and the cell moves in that direction.

In this hypothesis of the mechanism of chemotaxis it has been assumed that the response of the cell is excited by substances dissolved from the attracting particles, such as bacteria, or from the repelling particles of silicate. These substances in solution are supposed to form a concentration gradient about the cell. Though this is the obvious way to picture the relation between particles and cells and is perhaps the correct one, yet with this explanation certain observations are not in accord. Thus my co-workers and I have studied the chemotactic reactions of leukocytes to particles of collodion,⁴² of aluminum silicate⁷ and of carbon.⁴⁸ To the first of these, chemotaxis is positive, to the second negative, to the third indifferent. Yet all of these particles are practically water insoluble. How, then, can they form a concentration gradient? Nor does any amount of washing of these particles affect the chemotactic response as might be expected if the effects were due to impurities. These observations suggest the question whether chemotaxis may be a response not to a concentration gradient of substances in solution but to a field of some other nature which forms about the collodion, silicate or carbon particles.

SUMMARY

Chemotaxis may be defined as a directional response to a substance in the environment. The organism or cell is able to detect a concentration gradient of the substance and reacts by moving toward higher concentrations (positive chemotaxis) or toward lower concentrations (negative chemotaxis). Of cells displaying chemotaxis, the one of chief interest to pathologists is the leukocyte. Among mammalian leukocytes, chemotaxis has thus far been shown to be well developed only in the polymorphonuclears, while lymphocytes, though they move by the same mechanism, appear not to show chemotaxis. Mammalian monocytes also appear to lack positive chemotaxis and show the negative reaction only weakly.

Positive chemotaxis is of advantage in leading leukocytes directly toward bacteria and injured tissue, thus causing more cells to reach the

48. McCutcheon, M., and Dixon, H. M.: Unpublished data.

bacteria more quickly; the reaction brings the leukocyte into direct contact with the bacterium, thus giving opportunity for phagocytosis. Negative chemotaxis is as well developed as positive, but the usefulness of this reaction is uncertain.

Emigration of leukocytes from vessels can apparently occur without chemotaxis but is greatly increased when chemotactic substances are present. Immigration of leukocytes from sinuses of the bone marrow into blood vessels has been supposed to be due to chemotaxis, but the evidence for this supposition is unconvincing.

Chemotaxis in leukocytes was formerly thought to depend almost exclusively on forces operating on the cell surface. The view advocated in the present paper is that the reaction consists in the orientation of colloidal changes within the cell.

BIOCHEMICAL FACTORS IN INFLAMMATION AND DIABETES MELLITUS

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Studies of the basic factors which condition the cellular sequence in an area of acute inflammation have led to investigation of the mechanism involved in the enhancement of diabetes mellitus when this condition is complicated by superimposed inflammation.

For many years it has been known that the cytologic sequence in acute inflammation is characterized in the early stages by active emigration of polymorphonuclear leukocytes. After a time this gives way to infiltration by mononuclear phagocytes. The latter have been designated by various names, the most satisfactory of which is perhaps "macrophage," originally suggested by Metchnikoff. In acute inflammation the polymorphonuclear cells that leave the blood stream are the chief cellular constituents of the early exudate. The mononuclear phagocytes, or macrophages, increase in number in the later stages. These cells act as scavengers and when the inflammatory irritant has been overcome they engage actively in engulfing and digesting polymorphonuclear leukocytes, red cells and various necrotic materials resulting from the acute inflammation.

The orderly cytologic sequence whereby polymorphonuclear leukocytes precede the macrophages was first pointed out by Borrel,¹ in 1893, and then by Durham,² in 1897. The studies of Beattie³ extended considerably the original observations of Durham. This sequence holds for the majority of the inflammatory reactions caused by bacteria or by chemical irritants. The process has no direct reference to the actual migration of polymorphonuclear leukocytes into an area of injury. In earlier studies I pointed out that the latter phenomenon seems to be referable to the liberation of a nitrogenous substance termed leukotaxine.⁴ It is noteworthy that both tubercle and typhoid bacilli during the first twenty-four hours after their inoculation into normal tissues produce the same type of cellular changes, as do various forms of pyogenic

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1. Borrel, A.: *Ann. Inst. Pasteur* **7**:593, 1893.
2. Durham, H. E.: *J. Path. & Bact.* **4**:338, 1897.
3. Beattie, J. M.: *J. Path. & Bact.* **8**:129, 1903.
4. Menkin, V.: *J. Exper. Med.* **67**:145, 1938; *Physiol. Rev.* **18**:366, 1938.

bacteria, such as *Staphylococcus aureus* (Opie⁵; Vorwald⁶). The difference in the leukocytic response found with various types of inflammatory irritants seems, therefore, one of degree rather than of kind.

No adequate explanation has been offered for this fundamental process. A number of years ago various investigators, particularly Opie, studied the action of intracellular proteolytic enzymes from leukocytes of an inflammatory exudate.⁷ Müller,⁸ and also Opie, showed that polymorphonuclear leukocytes contain an intracellular enzyme that acts in a slightly alkaline or neutral medium but is almost wholly inactive in an acid reaction (0.2 per cent acetic acid). Opie designated this intracellular enzyme "leucoprotease." The action of this polymorphonuclear enzyme occurs only within the leukocyte, for in the plasma of an inflammatory exudate its activity is inhibited owing to the action of anti-enzymes. The earlier observations of Opie on the presence of antiferments inhibiting the action of leucoprotease have been confirmed by Weiss.⁹ Opie furthermore demonstrated that the mononuclear phagocytes that accumulate in the later stages of the inflammatory reaction contain an enzyme which causes active digestion of protein in a weakly acid medium but is almost entirely inactive at a neutral or an alkaline reaction. The enzyme of the mononuclear phagocyte has been called "lymphoprotease."

It is conceivable that particles in an inflammatory exudate prior to being phagocytosed by a given type of leukocyte may tend to have on their surfaces a hydrogen ion concentration approximating that at which an intracellular proteolytic enzyme is most capable of digesting them. If this assumption is correct, it is to be expected that the inflammatory exudate in which such particles are immersed would show a gradual increase in acidity concomitant with the shift from polymorphonuclear to mononuclear phagocytes. The question arises, therefore, as to whether or not there is a correlation between the p_H of the medium and the cytologic picture during the development of an acute inflammatory reaction. Lord, in his studies on proteolytic enzymes in the pneumonic lung, concluded that during the course of the disease a gradual increase in the hydrogen ion concentration of the exudate probably occurs.¹⁰ He conceived resolution to be the result of this increased hydrogen ion concentration, which eventually activated a proteolytic enzyme having a range of optimum reactivity at p_H 6.3 and 5.2.

5. Opie, E. L.: Arch. Int. Med. **5**:541, 1910.

6. Vorwald, A. J.: Am. Rev. Tuberc. **25**:74, 1932.

7. Opie, E. L.: J. Exper. Med. **7**:316, 1905; **8**:410, 1906; Physiol. Rev. **2**:552, 1922.

8. Müller, F., cited by Kossel, H.: Ztschr. f. klin. Med. **13**:149, 1888.

9. Weiss, C.: J. Infect. Dis. **41**:467, 1927.

10. Lord, F. T.: J. Exper. Med. **30**:379, 1919.

Rous¹¹ found that death of small cell aggregates resulted in the development of alkalinity in these cells, owing to seepage into them of alkaline body fluids. He recognized that the chemical changes that take place in small foci of necrosis differ in important respects from those occurring in large masses of dead tissue. He was led by his observations to conclude that very pronounced inflammatory edemas yield alkaline fluids but that "inflammation, as such, conduces to local acidosis." Schade and co-workers¹² reported that pus from acute abscesses had a p_H ranging from 5.95 to 6.50; the p_H of normal tissue fluids ranges from about p_H 7.10 to p_H 7.40.

None of the studies mentioned had correlated the p_H of the inflammatory exudate with the latter's differential leukocyte count. Several years ago, in studies on pleural effusion induced by turpentine, I¹³ obtained data on the trend of the hydrogen ion concentration. This was related to the cellular changes in the samples of exudate obtained at various intervals from the area of acute inflammation. The relation suggested that the prevailing hydrogen ion concentration may be an important factor in determining at a given time the cytologic picture of an inflammatory exudate.

In the experimental studies aforementioned I introduced turpentine (1.5 cc.) as an irritant into the pleural cavities of dogs. The p_H of the exudate and the corresponding cellular picture were studied from day to day. As the inflammatory reaction progressed, the reaction changed from an alkaline to an acid one. The change in the reaction toward definite acidity occurred usually two or three days after the injection of the irritant. Concomitant with this decrease in the alkalinity of the exudate there was a change in the differential leukocyte formula. The percentage of normal-appearing polymorphonuclears fell, whereas the percentage of mononuclear phagocytes correspondingly rose. The percentage of lymphocytes evidently played no significant role in these cellular changes. These observations have essentially little to do with the studies and interpretations of Hoff.¹⁴ This investigator stated that in acute inflammation there develops local acidosis with predominance of neutrophils but that in chronic inflammation with the appearance of lymphocytes the reaction tends to be alkaline. This at first seems to contradict my findings.¹⁵ Close scrutiny, however, indicates that Hoff

11. Rous, P.: *J. Exper. Med.* **44**:815, 1926.

12. Schade, H.; Neukirch, P., and Halpert, A.: *Ztschr. f. d. ges. exper. Med.* **24**:11, 1921. Schade, H.: *Die Molekularpathologie der Entzündung*, Dresden, Theodor Steinkopff, 1935.

13. Menkin, V.: *Am. J. Path.* **10**:193, 1934.

14. Hoff, F.: *Ergebn. d. inn. Med. u. Kinderh.* **46**:1, 1934.

15. (a) Menkin, V., and Warner, C. R.: *Am. J. Path.* **13**:25, 1937. (b) Menkin.¹³

failed to analyze the various stages involved in the gradual development of local acidosis at the site of inflammation. In the initial phase the exudate is at an alkaline p_H and there is predominance of polymorphonuclear leukocytes. With a fall in p_H these cells are still found in abundance, but they are degenerated or injured, whereas the macrophages appear relatively normal unless the p_H reaches a critical low level. At a p_H of about 6.0 all types of leukocytes are severely injured, resulting in pus formation. In the final phase of repair there is regeneration of vascular channels (i. e., granulation tissue). At this stage the reaction presumably tends to become alkaline. The relation, however, of this final phase to lymphocytic infiltration is unknown.

The results of my studies¹³ can be briefly restated as follows:

1. In the initial stage of an inflammatory reaction, the p_H of the exudate ranges from approximately 7.2 to about 7.3, and the polymorphonuclear leukocytes form the predominating cellular element.

2. With progress of the acute inflammation, there follows a rise in the hydrogen ion concentration. At a p_H of about 7.0 there are found about as many polymorphonuclears as mononuclear phagocytes.

3. With further reduction in p_H (6.9 to 6.8), the polymorphonuclear leukocytes are found injured or degenerated, whereas the macrophages appear to be relatively normal. When, however, the p_H reaches a level of about 6.5 or below, all forms of leukocytes reveal evidence of severe injury. At this stage the exudate is virtually that of suppuration. Pus, to a large extent, is therefore the outcome of an increase of local acidity causing severe injury and death of cells. The production of pus is consequently to be regarded, at least in part, as a function of the hydrogen ion concentration.

If the p_H remains at an alkaline level throughout the duration of inflammation, the polymorphonuclear leukocyte persists as the predominant cell type. Furthermore, studies have shown that the hydrogen ion concentration seems to be the conditioning factor in determining the cytologic picture at the site of acute inflammation rather than the reverse. As stated in an earlier paragraph, the mechanism seems to be primarily referable to the inability of polymorphonuclear leukocytes to survive in an acid medium; the macrophages display in this regard distinctly greater resistance. These studies have likewise been repeated and the observations confirmed by *in vitro* experiments.¹⁶ It has thus been shown that polymorphonuclear leukocytes are severely damaged when subjected to a buffer medium at p_H 6.6 or thereabouts. Macrophages, on the other hand, are apparently undamaged in a medium with p_H ranging between 6.5 and 6.8. At a lower p_H , however, such as 5.5, these cells are distinctly

16. Menkin, V.: Arch. Path. **27**:115, 1939.

injured. In brief, the evidence supports the view that the viability of leukocytes in an acutely inflamed area is a function of the local concentration of hydrogen ions. The macrophages are more resistant than the polymorphonuclear cells to changes in hydrogen ion concentration. The replacement of polymorphonuclear cells by macrophages in the course of inflammation is reasonably explained on the basis of the development of acidosis in the inflamed area. With further increase in the local concentration of hydrogen ions, both types of leukocytes are actively affected, and a state of suppuration follows. The studies indicate that the cytologic picture in an inflamed area can be predicted with reasonable accuracy from the p_H of the medium and vice versa.

In view of the foregoing observations an understanding of the mechanism of local acidosis in inflammation is doubtless of considerable importance. The studies of Irisawa¹⁷ and of Ito¹⁸ showed that lactic acid is present in pus. Gessler¹⁹ pointed out that the oxygen consumption and the metabolic rate are enhanced in an inflamed area. This state of affairs would doubtless favor the development of local acidosis unless properly compensated by an equally increased effective fluid circulation at the site of inflammation. In previous studies, however, I have pointed out that the local circulation in areas of intense injury is impaired. This is particularly manifested by the development of a lymphatic blockade and by the presence of a fibrinous network.²⁰ This state of affairs retards the dissemination of foreign substances or of micro-organisms from the site of an acute inflammation. It is conceivable that as acid metabolites are formed in an acutely inflamed area and as the local circulation becomes defective there may occur a concomitant rise in the hydrogen ion concentration of the exudate. The observations substantiate such a point of view.^{15a} The results of numerous experiments may be recapitulated briefly as follows:

1. With the development of an acute inflammatory reaction there is gradual depletion in the local alkali reserve. In other words, the carbon dioxide capacity of the cell-free exudate progressively diminishes. This is correlated with an increase in the hydrogen ion concentration and by a concomitant shift in the cytologic picture, as described, from a polymorphonuclear to a mononuclear phase.

2. The depletion in alkali reserve at the site of inflammation is referable to an increase in the rate of glycolysis. As the inflammatory reaction progresses in intensity, the concentration of lactic acid is considerably augmented, and the result is a localized lactic acid acidosis.

17. Irisawa, T.: *Ztschr. f. physiol. Chem.* **17**:340, 1893.

18. Ito, H.: *J. Biol. Chem.* **26**:173, 1916.

19. Gessler, H.: *Arch. f. exper. Path. u. Pharmakol.* **163**:456, 1932.

20. Menkin, V.: (a) *J. Exper. Med.* **53**:171, 1931; (b) *Arch. Path.* **12**:802, 1931; (c) *Dynamics of Inflammation*, New York, The Macmillan Company, 1940.

Incidentally (and this is of interest in view of subsequent findings to be discussed in connection with the recent studies on diabetes) an inflammatory exudate manifests greater glycolytic activity than blood, as indicated by a higher level of exudate lactic acid and a correspondingly lower concentration of exudate sugar.

3. The evidence shows that the mechanism of local acidosis in inflammation is therefore primarily referable to an increase in the rate of glycolysis and a concomitant depletion in the local alkali reserve. The cytologic picture in an area of acute inflammation appears to be conditioned by the local hydrogen ion concentration, which in turn depends on the rate of glycolysis and the reduction in alkali reserve. The significance of implications of these facts in determining the severity of an acute inflammation are obvious, for it is quite likely that some of the differences in histologic manifestations of various infectious lesions may be ascribed to disturbances in the intermediary carbohydrate metabolism of the affected tissue. Furthermore, it is conceivable that the relative absence of leukocytic migration in the late stages of inflammation may be referable to a local increased concentration of lactic acid. This acid has been shown by Gabritschewsky²¹ to be negatively chemotactic. Its formation in appreciable amounts may thus counteract the continuous migration of white cells induced by the liberation of leukotaxine.

DIABETES AND INFLAMMATION

The foregoing studies on some of the biochemical factors conditioning the cellular picture at the site of acute inflammation have led to investigation of a problem of definite significance to pathologists and clinicians. The effect of infection or inflammation on human diabetes is well known. In brief, the course of the disease is considerably accentuated, and to some extent the inflammatory reaction is intensified. There is also some evidence of a generalized fall in resistance as indicated by an augmented susceptibility to infection. The mechanism involved to explain the enhancement in the diabetic condition as well as the increased severity of the local inflammation have never been satisfactorily elucidated. Richardson²² in his studies was led to the conclusion that the enhanced susceptibility of diabetic persons to infection might be in part referable to a diminished capacity to form antibodies. I²³ have recently studied the problem. The results obtained indicate that the excessive hyperglycemia, which tends to develop in depancreatized dogs having a superimposed acute inflammation, seems referable to increased proteolysis at

21. Gabritschewsky, G.: *Ann. Inst. Pasteur* **4**:346, 1890.

22. Richardson, R.: *J. Clin. Investigation* **12**:1143, 1933; **14**:389, 1935; **19**:239, 1940.

23. Menkin, V.: (a) *Science* **93**:456, 1941; (b) *Am. J. Physiol.* **134**:517, 1941.

the site of inflammation. The deamination of the split protein molecule favors the formation of a surplus dextrose, which in turn diffuses into the blood stream. The increased local proteolysis results in severe damage of tissue, thus offering an explanation for the intensified inflammation. Both the enhanced protein catabolism and the increased formation of sugar in the inflamed area can be readily controlled by the administration of insulin.

The methods employed and the findings obtained^{23b} can be briefly listed as follows:

1. An attempt was first made to reproduce as closely as possible the clinical diabetic condition with superimposed inflammation. All experiments were made on depancreatized dogs. It is to be remembered in this connection that although there are many points of similarity between the human form of the disease and this type of experimental diabetes, nevertheless there are also some differences.²⁴ The inflammatory reaction was induced as in earlier experiments by the introduction of 1.5 cc. of turpentine into the pleural cavity. The results obtained indicate that following the superimposed and extensive inflammatory reaction there occurs a sharp ascent in the blood sugar level from an average of 253.0 mg. per hundred cubic centimeters to 469.1 mg. This is an average rise in blood sugar of 85.4 per cent. This increase can readily be inhibited by the administration of insulin. Furthermore, non-diabetic animals with pleural inflammation, as controls, fail to show any rise in blood sugar. In brief, the results seem to duplicate quite well the changes in blood sugar level that are observed in diabetic patients whose clinical course is complicated by infection.

2. An analysis was undertaken in an endeavor to unravel the basic mechanism which induces the sharp rise in the blood sugar of diabetic dogs having a superimposed pleural inflammation. Two preliminary considerations must first of all be borne in mind. One of the cardinal features of inflammation, which perhaps has not been sufficiently stressed, is the phenomenon of proteolysis. The early studies of Eichwald, published in 1864, on the presence of a peptone in pus, the observations of Friedrich Müller on the autolytic property of the purulent exudates of tuberculosis and pneumonia and finally the classic observations of Opie on the enzymatic property of leukocytes in digesting proteins support the view that proteolysis is an important process in the development of the inflammatory reaction. The studies²⁵ on the isolation of leukotaxine likewise substantiate this fact. By comparing the concentrations of amino acid nitrogen and of total proteins in exudates with the concentrations

24. Long, C. N. H., in *Harvey Lectures, 1936-1937*, Baltimore, Williams & Wilkins Company, 1937, p. 194.

25. Menkin, V.: *J. Exper. Med.* **67**:129, 1938.

of these same constituents in blood serum, the conclusion was drawn that proteolysis forms a conspicuous feature of the inflammatory reaction.

In the second place the conversion of part of the protein molecule to dextrose is a well recognized phenomenon. Claude Bernard believed in the possibility of such gluconeogenesis. The studies of numerous investigators cited elsewhere attested the truth of this principle.^{23b} Definite proof that in diabetes sugar originates in part from amino products was furnished by Stiles and Lusk.²⁶ Furthermore, the observations of Neuberg and Langstein²⁷ indicated that in normal rabbits the ingestion of alanine was followed by the appearance of lactic acid in the urine. Ringer and Lusk²⁸ found that in the phlorhizinized animal alanine is completely converted into dextrose. These various observations demonstrated that cleavage of proteins through deamination is followed by the formation of dextrose from part of the protein molecule.

In brief, the various studies cited indicate that in an inflamed area there is active proteolysis and that furthermore in the diabetic animal with increased protein catabolism (Lusk²⁹) dextrose can readily originate from amino products. Therefore, it seems reasonable to assume that in the focus of proteolysis in an inflamed area, the process of protein breakdown leading to gluconeogenesis may be considerably enhanced if the inflamed area is in a diabetic animal. It probably is quite immaterial whether one abides by the nonutilization or the overproduction theory of diabetes.³⁰ In either case, gluconeogenesis could occur at the site of inflammation, which in this particular aspect would function as a sort of accessory liver. The excess dextrose thus formed would gradually diffuse into the circulating blood. The observations apparently substantiate this concept.

3. The carbohydrate and protein metabolism in the exudates of depancreatized and nondiabetic dogs was investigated by studying the respective concentrations of sugar, lactic acid, total proteins, nonprotein nitrogen, urea, amino acid nitrogen and the hydrogen ion concentration. These studies were further correlated with the cytologic picture of the exudate. The results indicated that in the exudates of diabetic animals, besides an elevation in the sugar and in the lactic acid content, there was likewise a marked rise in the products of protein breakdown.

The averaged results on the carbohydrate and nitrogenous constituents studied are conveniently shown in table 1. Furthermore, the

26. Stiles, P. G., and Lusk, G.: *Am. J. Physiol.* **9**:380, 1903.

27. Neuberg, C., and Langstein, L.: *Arch. f. Physiol.*, 1903, supp., p. 514.

28. Ringer, A. I., and Lusk, G.: *Ztschr. f. physiol. Chem.* **66**:106, 1910.

29. Lusk, G.: *The Science of Nutrition*, Philadelphia, W. B. Saunders Company, 1923.

30. Soskin, S.: *Physiol. Rev.* **21**:140, 1941.

averages of the results obtained on depancreatized dogs treated continuously with low doses of insulin are likewise listed. The lactic acid content of exudates is 52 per cent higher in diabetic than in nondiabetic animals (table 1). The administration of insulin to depancreatized animals completely inhibits the rise of lactic acid in exudate, the average level being kept down to 57.84 mg. per hundred cubic centimeters (table 1). The concentration of sugar in the exudates of diabetic dogs shows an increase of 473.6 per cent over that in the exudates of nondiabetic dogs. The level of sugar is distinctly reduced with repeated administration of insulin, the average being 191.7 mg. per hundred cubic centimeters.

The observations regarding the status of protein metabolism in exudates are of even greater interest. The average value of the total proteins per hundred cubic centimeters of exudate in diabetic dogs is

TABLE 1.—*Composite Data on the Metabolism of Carbohydrate and of Protein in Exudates of Diabetic Dogs, Nondiabetic Dogs, and Depancreatized Dogs Treated With Low Doses of Insulin*

Source of Pleural Exudates	Average Concentration					
	Lactic Acid, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Total Proteins, Gm. per 100 Cc.	Nonprotein Nitrogen, Mg. per 100 Cc.	Urea, Mg. per 100 Cc.	Amino Acid Nitrogen, Mg. per 100 Cc.
Nondiabetic dogs.....	58.75	78.87	4.54	42.56	34.5	7.39
Diabetic dogs.....	89.29	452.4	3.97	80.63	78.1	12.88
Depancreatized dogs treated with low doses of insulin..	57.84	191.7	4.49	32.3	21.5	8.0

12.56 per cent lower than that encountered in nondiabetic animals (table 1). In the insulin-treated group the total protein concentration is approximately that found in the nondiabetic dogs. The products of protein catabolism show even much more striking differences in the three groups of animals (table 1). In the diabetic exudate there is an increase of 89.45 per cent in the nonprotein nitrogen concentration. The urea level shows a rise averaging 126.3 per cent. The amino acid nitrogen in the exudates of diabetic dogs likewise shows an increase of 74.29 per cent over the level found in the exudates of nondiabetic animals. These figures indicate a markedly enhanced degree of proteolysis at the site of inflammation in depancreatized dogs.

4. It is well known that insulin inhibits the formation of dextrose from noncarbohydrate precursors.³¹ If the excess sugar formation in the

31. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, Williams & Wilkins Company, 1932, vol. 1, p. 301. Jensen, H. F.: *Insulin: Its Chemistry and Physiology*, New York, Commonwealth Fund, Division of Publications, 1938.

inflamed area is primarily derived from the local breakdown of proteins, the administration of insulin should both depress the level of dextrose and inhibit the enhanced protein catabolism. This is precisely what occurred (table 1). In other words, injections of insulin reduced not only the level of sugar and of lactic acid in diabetic exudates but diminished as well the local proteolysis. This fact supports the view that gluconeogenesis at the site of acute inflammation in a diabetic animal originates from proteins through deamination of the molecule. A further, though perhaps indirect, type of evidence was also obtained for arriving at this conclusion. As pointed out in the earlier part of this discussion, it has been shown that the conversion of sugar to lactic acid is the primary factor responsible for the gradual increase in hydrogen ion concentration at the site of acute inflammation.^{15a} The observations have revealed a reciprocal type of relation between the concentrations of sugar and lactic acid in exudate.^{15a} On the other hand, in the inflamed area of a diabetic animal no such consistent reciprocal relation is detectable, indicating that in all probability the level of lactic acid in the diabetic exudate is not wholly conditioned by the actual concentration of sugar.^{23b} The parallelism in the levels of lactic acid and sugar occasionally encountered in a diabetic exudate suggests rather that this state of affairs may well be referable to a direct origin of these respective substances from products of protein breakdown.

5. The biochemical changes encountered in the exudate of a depancreatized dog were similarly reflected in the circulating blood. Besides an elevation in blood sugar level, there was found likewise a marked increase in the blood concentrations of nonprotein nitrogen, urea and amino acid. A summary of the actual data is shown in table 2. The details of these observations have been reported elsewhere.^{23b} Pancreatectomy per se failed to induce any significant alteration in the nitrogenous constituents of the circulating blood (table 2). A comparison of the relative concentrations of dextrose in exudate and blood revealed that the sugar level of exudate was distinctly higher than that of blood, indicating that the surplus sugar is doubtless formed at the site of inflammation from which it gradually diffuses into the blood stream rather than the reverse. The blood sugar averaged 447.7 mg. per hundred cubic centimeters, whereas the exudate yielded an average of 532.0 mg. per hundred cubic centimeters. The higher exudate sugar in diabetic animals occurred despite the fact that the glycolysis in an inflamed area is more active than that in the blood.^{15a} For instance, in nondiabetic dogs the exudate sugar averaged 63.5 mg., compared with 81.8 mg. per hundred cubic centimeters in the blood. On the other hand, it is interesting to note that the enhanced local gluconeogenesis from pro-

teins in the inflamed area of a diabetic animal evidently transcends the active glycolytic process, so that the exudate sugar is still higher than that of blood.

As shown in table 2, the presence of acute pleural inflammation in a nondiabetic dog failed to alter appreciably the concentration of either carbohydrate or nitrogenous metabolic products in the circulating blood. Continuous administration of insulin in diabetic animals with superimposed inflammation likewise repressed the excessive hyperglycemia and the tendency toward azotemia.^{23b} The heightened concentrations of carbohydrate and nitrogenous products in the blood of the diabetic

TABLE 2.—Composite Data on the Carbohydrate and Nitrogenous Constituents in the Circulating Blood of Nondiabetic and Depancreatized Dogs with Inflammation

Source of Blood Samples	Time of Withdrawal of Blood	Average Concentration					
		Lactic Acid, Mg. per 100 Ce.	Sugar, Mg. per 100 Ce.	Total Proteins, Gm. per 100 Ce.	Nonprotein Nitrogen, Mg. per 100 Ce.	Urea, Mg. per 100 Ce.	Amino Acid Nitrogen, Mg. per 100 Ce.
Nondiabetic dogs	Either prior to or inducing pleural inflammation or before pancreatectomy.....	14.16	84.16	6.33	35.18	6.56
	When there was a concomitant pleural inflammation of varying duration.....	16.74	90.0	5.46	35.26	21.9	5.85
Diabetic dogs	Either prior to or inducing pleural inflammation or before pancreatectomy.....	16.16	96.87	5.67	39.3	37.75	6.87
	Several days after pancreatectomy.....	20.61	265.51	5.04	34.14	21.75	9.54
	When there was a concomitant pleural inflammation of varying duration.....	30.96	473.81	4.53	85.14	70.2	10.54

dog are evidently referable to absorption from the inflamed area. This in no way contradicts my earlier findings on the fixation of various materials in an acutely inflamed area.²⁰ The degree of fixation is referable to the intensity of local injury and also to the size of the particle.³² For instance, it has been demonstrated that proteins are less readily retained than bacteria or graphite particles.³³ Miller³⁴ has essentially substantiated this concept by demonstrating that diffusible substances are rapidly absorbed from an inflamed area.

6. The observations on the exudate of depancreatized dogs indicate that there is enhanced proteolysis at the site of inflammation. This fact

32. Menkin, V.: J. Exper. Med. **57**:977, 1933; footnote 20 c.

33. Menkin, V.: J. Exper. Med. **52**:201, 1930.

34. Miller, R. G.: J. Exper. Med. **67**:619, 1938.

of itself would entail increased cellular damage. It is therefore conceivable that the mechanism responsible for enhancing the course of experimental diabetes might simultaneously explain the accompanying increase in severity of the inflammatory reaction. The lactic acid content of exudates is considerably higher in diabetic than in nondiabetic animals (table 1). Concomitantly, the p_H tends to be lower, and the cellular picture, in accordance with the studies described at the beginning of this discussion, is thereby affected. In a one day exudate the polymorphonuclear leukocytes are not always found to be the predominating cell type. Furthermore, in contrast to the picture in the exudate of nondiabetic controls, the leukocytes usually appear injured, vacuolated or degenerated. These morphologic features add further support to the chemical findings of increased local proteolysis at the site of inflammation in a diabetic animal.^{23b}

The foregoing observations and the inferences drawn raise a number of questions and problems. For instance, what regulating process in the diabetic organism actually enhances local proteolysis in an inflamed area? The role of various endocrine structures, such as the adrenals and the pituitary, or even possibly of the liver, is now being investigated. Is gluconeogenesis from proteins in a diabetic animal restricted only to inflammatory foci, to the liver and to the kidneys? It is conceivable that this phenomenon may perhaps occur in any area where there is conspicuous tissue damage such, for instance, as is encountered in an arteriosclerotic lesion. The clinical and pathologic importance of this problem is obvious and therefore needs no further comment. Although gluconeogenesis from noncarbohydrate precursors is known to occur in the liver and the kidneys, it is a new concept that such a process can occur at the site of inflammation in a diabetic animal. For this reason, further studies are now in progress in an attempt to determine in a more direct and precise manner whether such formation does take place locally in an injured area of a depancreatized dog.³⁵ To what extent

35. Numerous additional observations have been made since this paper was sent to the ARCHIVES, which fully substantiate the original view that gluconeogenesis definitely occurs in the acutely inflamed area of a diabetic dog. For instance, if the sugar concentrations in the exudate and the blood are compared at frequent intervals after the introduction of an irritant into the pleural cavity, it is found that in the first few hours of inflammation the exudate sugar is considerably higher than the blood sugar. The latter, in fact, shows at first no enhancement of its level. It is only after the inflammation has progressed for about a day that the excessively high sugar level in the exudate becomes in part reflected in the circulating blood. The establishment of this definite gradient between exudate and blood sugar level from the earliest stages of the inflammatory reaction (i. e., as early as one hour) strongly supports further the view that local gluconeogenesis occurs at the site of inflammation in a diabetic dog. These recent observations will be reported in extenso elsewhere.

does diabetes interfere with the normal synthesis of protein, and what effects may this interference, if it occurs, have on various immunologic phenomena such as antibody production? Finally, it is highly important to extend the present observations on experimental animals to human diabetic patients whose disease is complicated by severe inflammatory processes.

SUMMARY AND CONCLUSIONS

The usual cellular sequence in an area of acute inflammation is characterized by an initial infiltration of polymorphonuclear leukocytes, which are subsequently replaced by an abundance of macrophages. The underlying conditioning mechanism concerned in this basic phenomenon seems to be the production of local acidosis. Above p_H 7.0 the polymorphonuclear leukocytes predominate. With progressive increase in the intensity of the inflammatory reaction there is concomitant increase in the hydrogen ion concentration. Under such circumstances the polymorphonuclear leukocytes are unable to survive in a medium of p_H 6.8 or thereabouts, while, on the contrary, the macrophages appear uninjured. Below a p_H of about 6.5 all types of leukocytes are affected and a state of suppuration ensues. Thus the formation of pus is to a large extent a function of the hydrogen ion concentration. The hydrogen ion concentration as observed by *in vivo* and *in vitro* methods determines the survival of leukocytes, but the diapedesis of polymorphonuclear leukocytes into inflamed tissue is referable to the liberation of leukotaxine. Migration is therefore not to be considered as directly related to the hydrogen ion concentration.

The production of local acidosis in an acutely inflamed area is referable to a depletion in the alkali reserve. This, in turn, appears to be due to a disturbance in the intermediary carbohydrate metabolism in the form of increased glycolysis, which favors the production of a true lactic acid acidosis.

The studies reported here are of significance in aiding understanding of some of the biochemical systems that condition the histologic manifestation of various infectious lesions.

The results have led to a subsequent study on the mechanism of the enhanced diabetes in depancreatized dogs with a superimposed acute inflammation. The mechanism, in brief, seems to be primarily referable to augmentation of the proteolysis at the site of inflammation. The products derived from this increased protein catabolism seem responsible for a marked degree of local gluconeogenesis. The surplus dextrose apparently derived from the degradation of the protein molecule in the injured area gradually diffuses into the systemic circulation, elevating further the blood sugar level. The administration of insulin seems to

repress both the increased proteolysis as measured in the exudate and the accompanying gluconeogenesis. This fact suggests the rationale of intensive insulin therapy in diabetes complicated by infection. The increase in local protein breakdown in the inflamed area of a diabetic animal implies increased tissue damage. This is substantiated by the appearance in such exudates of leukocytes which reveal pronounced signs of cellular injury when compared with similar cells from a non-diabetic exudate. The enhanced local proteolysis in the inflamed area suggests a reasonable explanation for the increased tissue damage and the corresponding elevation in the dextrose level. Further studies are in progress in an endeavor to obtain additional information on the apparent process of gluconeogenesis at the sites of inflammation in the diabetic animal.

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EFFECT OF PARABIOSIS ON THE HEPATIC
CHANGES FOLLOWING OBSTRUCTION OF
THE COMMON BILE DUCT IN RATS

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The cause of the pathologic changes in the liver that follow obstruction of the common bile duct—necrosis of the hepatic cells, fibrosis in the portal areas and proliferation or elongation of the bile ducts—is not completely understood. It was early thought that these lesions were found only if infection was present,¹ but recent studies by Lieber and Stewart² and by McMahon and Mallory³ clearly show that there is in man a definite entity of obstructive biliary cirrhosis in the absence of infection. Following obstruction of the extrahepatic bile ducts, the smaller bile ducts are dilated and are filled with an inspissated brown material. It has been generally assumed that the retained bile is largely responsible for the necrosis of the hepatic cells and that the dilatation of the bile ducts and the presence of the inspissated substances bring about the epithelial proliferation and fibrosis. These conclusions are based on the known toxic action of bile salts and on a morphologic study of the liver in man and in experimental animals, neither of which is definitive. Our experiments show that the procedure of parabiosis offers a method for the separation of the two factors—retention of bile and dilatation. If the common bile duct of a rat united in parabiotic union with another rat is ligated, the biliary system undergoes conspicuous dilatation and is filled with a clear viscid fluid, but there is no retention of bile pigments in the ducts or in the liver and no demonstrable jaundice. The liver of the parabiotic twin apparently secretes the biliary constituents for both animals.

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1. Opie, E. L.: *J. A. M. A.* **85**:1533, 1925.

2. Lieber, M. M., and Stewart, H. L.: *Arch. Path.* **17**:362, 1934.

3. McMahon, H. E., and Mallory, F. B.: *Am. J. Path.* **5**:645, 1929.

PROCEDURE

White rats, weighing about 200 Gm. each, were united in parabiosis by the usual technic. With the animals under ether anesthesia, the skin on the lateral wall of the thorax and abdomen was incised. The ventral skin edges were sewn with a subcuticular continuous stitch. The peritoneal cavities were then opened and the edges sutured. In some animals the scapulas were pulled together and sutured. Finally the dorsal skin edges were sewn. A broad band of adhesive tape about the thoraxes prevented the animals from pulling apart and placing tension on the wound. On the fourth day the existence of a collateral circulation was demonstrated by the injection of phenolsulfonphthalein into one animal and recovery of it in the urine of the other. On the fifth day the twins were anesthetized with

Summary of Experiments

Parabiotic Twins								Controls				
Animal	Days Obstructed	With Obstruction			Normal			Animal	Days Obstruction	Fibrosis	Dilatation and Proliferation	
		Fibrosis	Dilatation and Proliferation	Necrosis	Fibrosis	Dilatation	Necrosis				Fibrosis	Necrosis
J-3	2	0 *	0	+	0	+	0	J-20	2	0	++	++
J-6	3	0	+	+	0	0	0	J-12	3	0	+	++
581	4	0	0	+	0	+	++	J-13	4	0	++	++
H-4	7	0	+	0	0	0	0	J-14	7	+	+++	+
585	7	0	+	+++	0	0	0	J-19	7	+	+	++
579	7	±	±	0	0	0	+++	J-26	7	+	++	0
583	7	+	++	+++	+	0	+++	J-27	7	+	++	++
M-3	12	++	0	+	0	0	0	J-18	13	+++	+++	0
H-6	14	0	+	+	0	0	0	J-28	13	+	+	+++
M-1	15	±	++	0	0	±	0	H-19	14	++	++	+
M-2	15	+	0	+	0	0	0	J-22	14	++	++	++
H-2	23	+	++	0	0	0	0	H-26	23	++	+	+
J-7	23	++	++	++	0	0	0	J-10	24	++	+	+
J-1	36	+++	++	+	0	0	0	J-32	36	++	+++	+
J-5	36	+++	++	+	0	±	0	J-33	36	++	++	+

* The signs 0, ±, +, ++ and +++ indicate varying degrees of change (fibrosis, dilatation, necrosis) from normal (0) to the most advanced observed in these experiments (+++).

ether and the common bile duct of the right hand member of the pair was doubly ligated and divided through a high right rectus incision. The bile ducts of single control animals were ligated in the same manner. At selected intervals after the second operation, pairs and control animals were killed and sections prepared from not less than three parts of the liver. In order to eliminate autolytic changes in the histologic evaluation, animals that died are not included in the report. From a few animals (all except those labeled J in the table) a small piece, 1 Gm., of liver was taken under sterile precautions, ground in a mortar and cultured in dextrose brain broth. Animals which had infection of the surgical wounds or from whose livers organisms were cultured are not included in this report.

OBSERVATIONS

Inspection of the table shows that up to fifteen days there occurred definitely more fibrosis and proliferation of the bile ducts in the control animals than in the parabiotic twin with obstruction of the bile duct.

Although the necrosis in the control animals was more conspicuous and extensive than in the experimental animals, the differences were not striking. There was no demonstrable necrosis in 3 control and 2 experimental animals. The cause of the necrosis in the 3 normal parabiotic twins is not clear, unless it was related to the overactivity of the livers of these animals, similar to the cloudy swelling of the renal epithelium following unilateral nephrectomy. After the twenty-third day, 3 of 4 animals showed the same degree of pathologic change. From these results it appears that the necrosis associated with uncomplicated obstruction of the common bile duct is an immediate reaction to the dilatation of the ducts. We did not observe actual rupture of the smaller ducts, such as has been reported by others, and therefore have no theory to offer for the causal relation between dilatation and necrosis. The presence of retained bile increases the severity of the necrosis but is not a necessary factor.

The fibrosis and proliferation of the ducts are a combined type of reaction. Up to fifteen or twenty days the changes are inconspicuous or absent in animals with obstruction but without jaundice and present in animals with obstruction and jaundice. After twenty days the lesions in the two types of obstruction are of about equal severity. These results suggest that the earlier phases of fibrosis and proliferation of the bile ducts are associated with the retention of bile while the later changes are related to simple dilatation.

SUMMARY

When 2 rats are joined by parabiosis, products of biliary secretion are eliminated by the normal member of the parabiotic pair after ligation of the common duct of the other animal. There is no accumulation of bile pigments and no jaundice, yet the ducts become dilated with clear, colorless secretion. Necrosis of the liver occurs in both parabiotic and control animals but is somewhat less in the former. Fibrosis subsequently takes place, though less promptly than in control animals with ligated ducts and obstructive jaundice. After fifteen days, however, no noteworthy difference in this particular can be discerned.

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BACTERIAL MORPHOLOGY AS SHOWN BY THE ELECTRON MICROSCOPE

IV. STRUCTURAL DIFFERENTIATION WITHIN THE BACTERIAL PROTOPLASM

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The differentiation of bacterial cell wall from inner fluid or potentially fluid protoplasm has been demonstrated in earlier studies with the electron microscope from these laboratories.¹ The pictures presented here have been selected because they show, in addition, structural differentiation within the bacterial protoplasm itself. These micrographs are offered as pure exposition, suggestive, however, of the possibilities of analytic studies directed toward eventual elucidation of the nature and significance of the differentiations within the protoplasm so vividly shown by the electron microscope.

The bacteria were grown under ordinary cultural conditions and were suspended in distilled water which had been passed through a sintered glass filter; a droplet of this suspension was allowed to dry on a collodion film mounted as previously described.² This preparation was then introduced into the evacuated chamber of the electron microscope, a suitable field found and a picture taken by a few seconds' exposure to the electron

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1. (a) Mudd, S., and Lackman, D. B.: *J. Bact.* **41**:415, 1941. (b) Mudd, S.; Polevitzky, K.; Anderson, T. F., and Chambers, L. A.: *ibid.* **42**:251, 1941. (c) Mudd, S.; Polevitzky, K.; Anderson, T. F., and Kast, C. C.: *Bacterial Morphology as Shown by the Electron Microscope: III. Cell-Wall and Protoplasm in a Strain of Fusobacterium*, *ibid.*, to be published. (d) Mudd, S., and Anderson, T. F.: *J. Immunol.* **42**:251, 1941.

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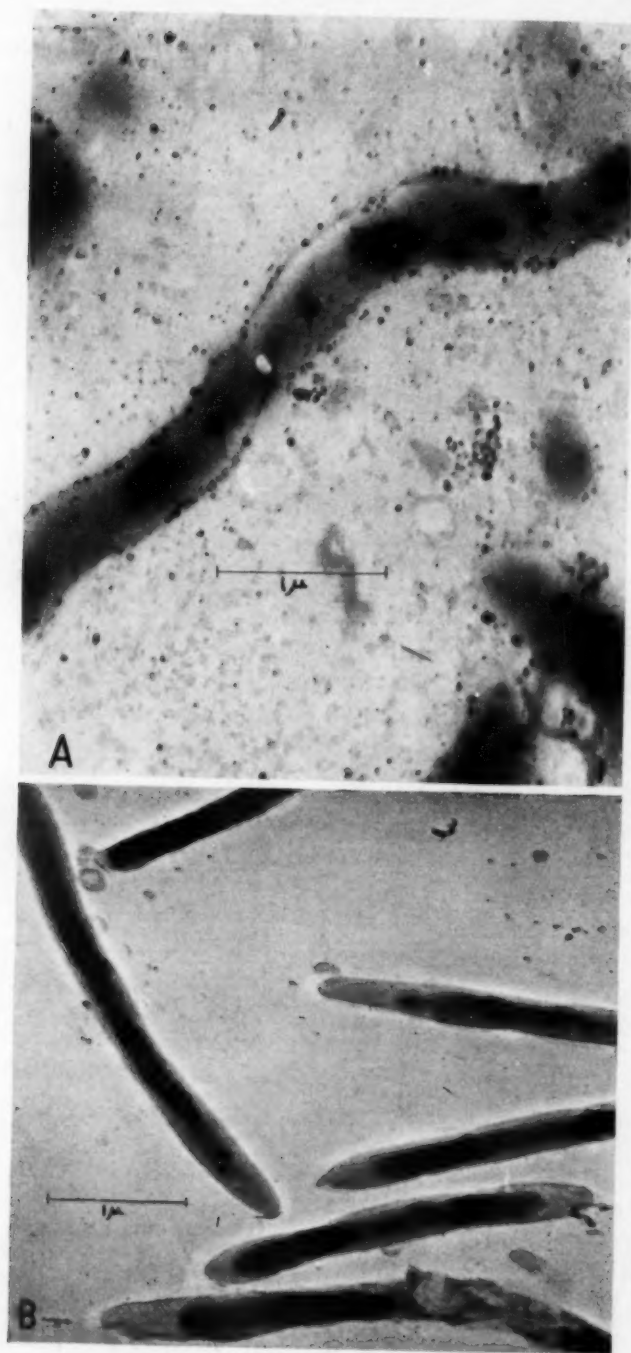


Figure 1

(See legend on opposite page)

beam. No fixation other than that of the drying and electron bombardment and no staining procedures were employed.

Figure 1 *A* shows cells of *Mycobacterium tuberculosis* (*hominis*). The cell wall appears to be very delicate. The many minute dark granules which are seen throughout the field and in particular adhering to the cell walls have been characteristic of pictures of tubercle bacilli. The thirteen largest of the black granules seen within the protoplasm range from 70 to 230 millimicrons (± 10 per cent) in diameter. Since the darkness of the image on an electron micrograph is, to a first approximation, proportional to the product of the thickness by the density of the object³ these granules must be far more dense than the protoplasm in which they are embedded. The granules in tubercle bacilli were described in Koch's original publications⁴ and have been described and interpreted by countless workers since, without, however, any one of the various interpretations having become established.⁵ An electron-micrographic study of tubercle bacilli has recently been published by Lembke and Ruska.⁶

Figure 1 *B* shows cells of a strain of *Fusobacterium*. Striking differences in the density of the protoplasm are apparent, but, in contrast to the micrograph of the tubercle bacilli, the dense areas shown are not localized in definite, circumscribed granules. The morphology of a strain of *Fusobacterium* has recently been presented.^{1c}

3. Anderson, T. F.: *The Study of Colloids with the Electron Microscope*, in *Advances in Colloid Science*, New York, Interscience Publishers, Inc., 1942.

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EXPLANATION OF FIGURE 1

A, *Mycobacterium tuberculosis* (*hominis*). The specimen was from a six week culture on glycerol-agar and was received from Dr. Esmond R. Long, of the Henry Phipps Institute of the University of Pennsylvania. A loopful of growth was transferred to a dry test tube containing glass beads and rubbed with a glass rod to break up clumps. The bacteria were then suspended in distilled water, and a droplet of the suspension was dried on a mount. Electron voltage 55 kilovolts. Magnification as here reproduced $\times 23,000$.

B, *Fusobacterium*. The specimen was from a forty-eight hour culture in cysteine-ascitic fluid broth medium and was received from Dr. C. C. Kast. 60 kilovolts. Magnification as reproduced $\times 18,500$.

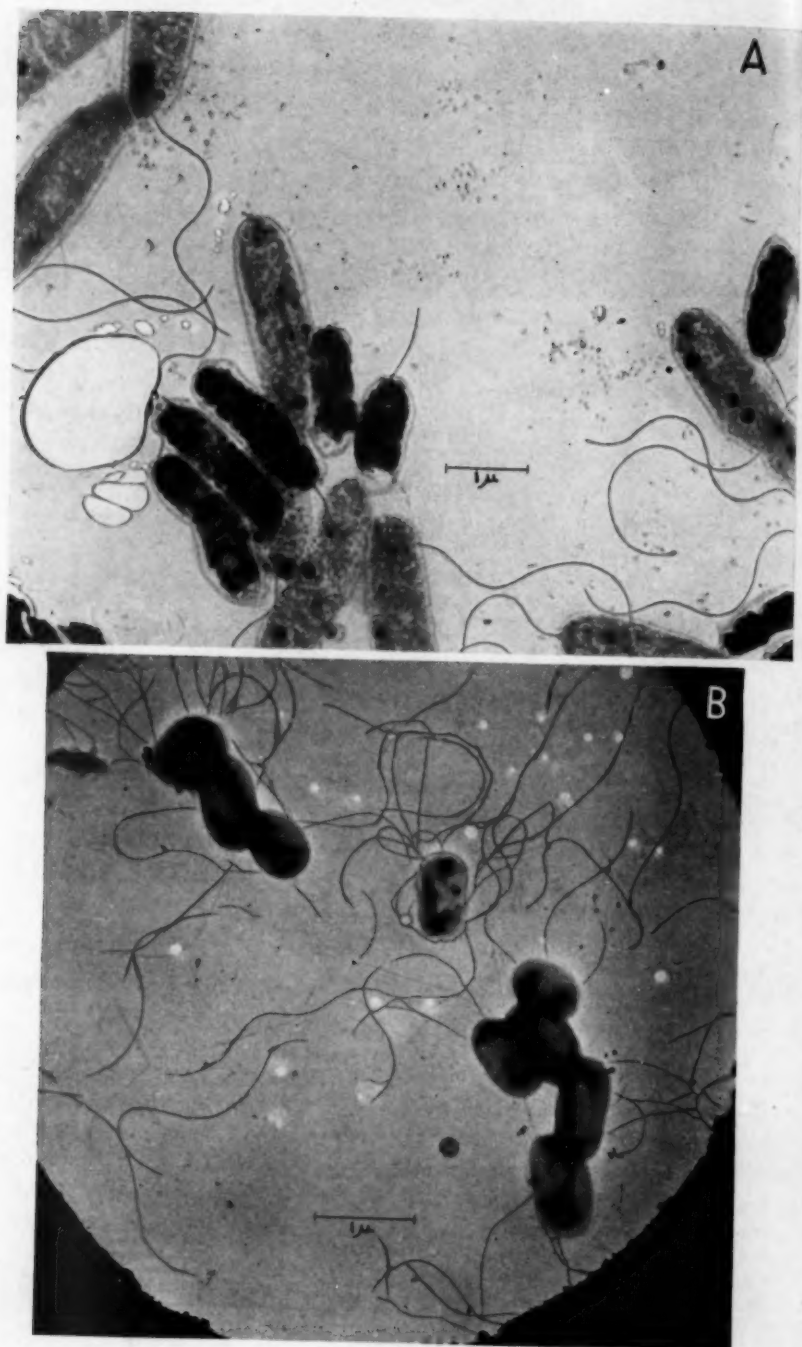


Figure 2

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Figure 2 *A* shows cells of *Vibrio schuylkiliensis*. Definite, circumscribed granules are again seen within the protoplasm. The nineteen most definite of these range in diameter from 90 to 230 millimicrons (± 10 per cent), with an average value of about 150 millimicrons. In addition to having discrete granules, the whole bacterial protoplasm appears to be either finely or relatively coarsely granular; this appearance is in our experience rare and may be a coagulation artefact due to drying or to the electron bombardment. The protoplasm is obviously shrunken away from the relatively "transparent" cell wall in various cells.

The monotrichate flagella of the vibrios are thicker than the peritrichate flagella of *Eberthella typhosa* (figs. 2 *B* and 3 *A*). According to Neumann,⁷ the flagella of monotrichate species are in general larger in diameter than those of peritrichate and lophotrichate species.

In electron micrography of objects the size of bacteria the depth of focus is considerably greater than the thickness of the object, so that an image of the entire three dimensional object is seen projected on the plane of the photographic plate.⁸ Thus the first impression given from inspection of figure 2 *A* that the flagella traverse the bacterial protoplasm is obviously due to the projection of flagella which actually are above or below and outside of the bacterial cell. On the other hand, at several points the pictures appear to indicate that the flagellum is continuous with the protoplasm. No basal granule is discernible at the origin of the flagellum.

Figure 2 *B* shows cells of *E. typhosa* and one ghost cell, or cell wall, from which most of the protoplasm has escaped.

7. Neumann, F.: *Centralbl. f. Bakt. (Abt. 1)* **109**:143, 1928.

8. Burton, E. F., and Kohl, W. H.: *The Electron Microscope*, New York, Reinhold Publishing Company, 1942. Marton.^{2a} Morton.^{2b} Anderson.³

EXPLANATION OF FIGURE 2

A, *Vibrio schuylkiliensis*. The specimen was from a twenty-four hour culture in nutrient agar. 100 kilovolts. Magnification as reproduced $\times 11,000$.

B, *Eberthella typhosa*. The specimen was from a twenty-four hour culture on nutrient agar. The organisms were suspended in 0.85 per cent sodium chloride solution and were washed on the mount in distilled water. 60 kilovolts. Magnification as reproduced $\times 13,000$.



Fig. 3.—*A*, *Eberthella typhosa*. The specimen was from a twenty-four hour culture on nutrient agar. The organisms were suspended directly in distilled water. 100 kilovolts. Magnification as reproduced $\times 18,000$. *B*, *Corynebacterium diphtheriae*. The specimen was received from Dr. Harry E. Morton. It was from a culture on blood agar, incubated forty-eight hours, then kept at room temperature for three days. 60 kilovolts. Magnification as reproduced $\times 17,000$.

Figure 3 *A* shows other cells of *E. typhosa* micrographed at higher voltage. Areas of greater density are seen at the poles of one cell but not of another. In the case of the dividing cell, a denser area is seen at one pole and a minute dense area at the other; in no case is the dense protoplasm localized in a discrete, definite granule.

Figure 3 *B* shows cells of *Corynebacterium diphtheriae*. Definite granules are seen at the poles of the cells. These granules range in diameter from 120 to 180 millimicrons (± 10 per cent) (Morton and Anderson⁹).

Figure 4 *A* shows two cells of *Spirochaeta pallida* (Noguchi strain). These appear not quite to have completed transverse division. Two very definite granules about 140 millimicrons in diameter are seen in the lower cell. Structures which resemble flagella are seen in this picture and have been regularly observed in electron micrographs of spirochetes. These will be discussed in a subsequent publication.

Remarkable structural differentiation within the protoplasm of a sulfur-oxidizing bacterium is recorded by Umbreit and Anderson.¹⁰

It may again be emphasized that the foregoing pictures have been selected because they do show structural differentiation within the bacterial protoplasm. Examples of a much larger number of pictures which do not show much differentiation within the protoplasm are given in figures 4 *B* and *C*. These are cells of *Lactobacillus acidophilus* in various stages of cell division. The cell wall appears as a light shadow enclosing dark protoplasm in which little variation in density is discernible.

For all the pictures herein reproduced, taken with 55 or 60 kilovolt electrons, the ordinary commercial model of the RCA electron microscope was used.¹¹ For the pictures at 100 kilovolts the new high voltage research instrument was used, through the kindness of Dr. V. K. Zworykin.¹²

9. Morton, H. E., and Anderson, T. F.: *Proc. Soc. Exper. Biol. & Med.* **46**: 272, 1941.

10. Umbreit, W. W., and Anderson, T. F.: *A Study of Thiobacillus Thio-oxidans with the Electron Microscope*, *J. Bact.*, to be published.

11. Zworykin, V. K.; Hillier, J., and Vance, A. W.: *Electrical Engineering* **60**:157, 1941.

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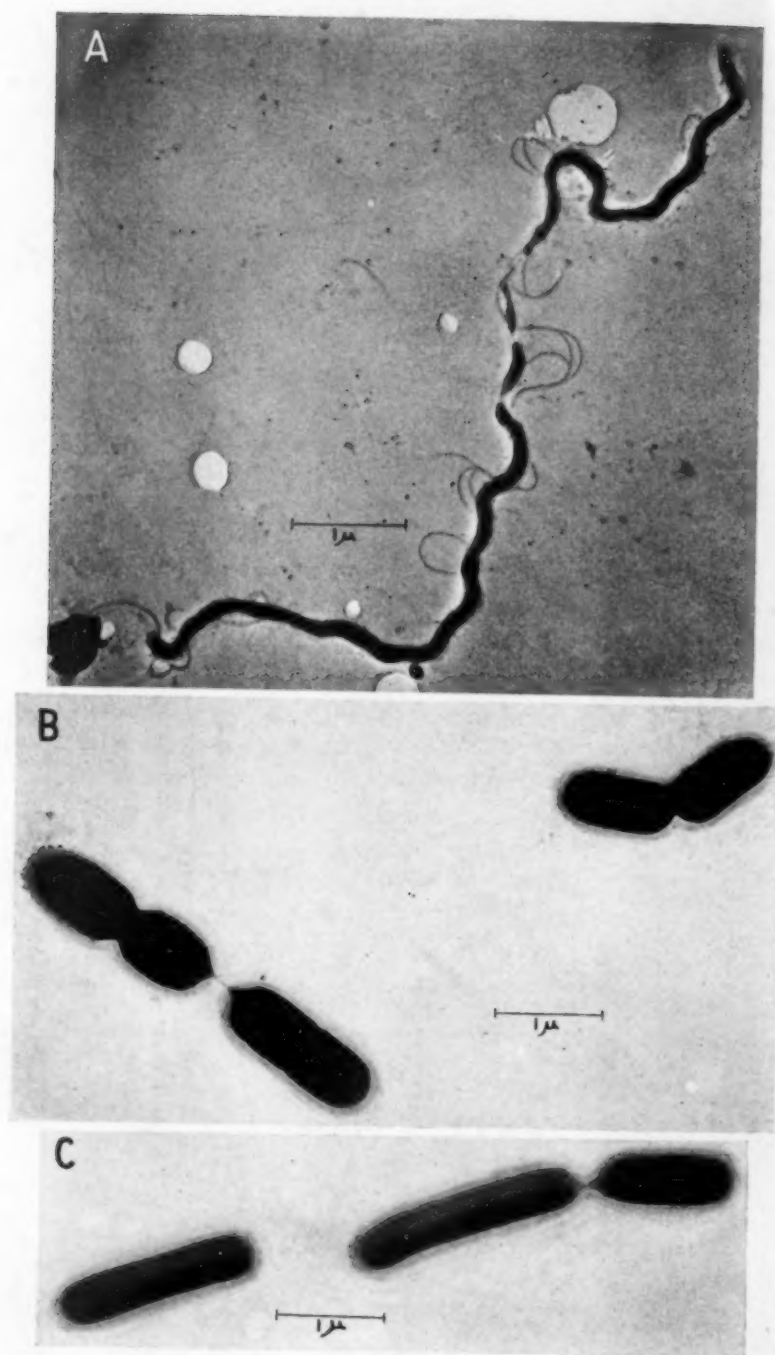


Figure 4

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SUMMARY

Electron micrographs of bacterial cells have clearly brought out the differentiation of solid bacterial cell wall from inner fluid or potentially fluid protoplasm. Within the inner protoplasm itself inhomogeneities of at least two kinds may be seen:

1. Discrete dense spheroidal or discoidal granules. Examples of these granules, ranging in diameter from 70 to 230 millimicrons (± 10 per cent), are shown in cells from the genera *Mycobacterium*, *Corynebacterium*, *Vibrio* and *Spirochaeta*.

2. Dense areas of markedly differing size, shape and position, not sharply circumscribed, are shown in cells of *Eberthella typhosa* and *Fusobacterium*.

Granules in bacterial protoplasm have been variously interpreted as nuclei or nuclear equivalents, reserve food material, reproductive elements, and otherwise,¹³ without any of these interpretations having become established. The electron microscope shows fine structure within bacteria with clarity and detail not hitherto possible, and when coordinated with cultural and cytologic procedures, should contribute to eventual understanding of these fine structures.

University of Pennsylvania Medical School.

13. Lewis, I. M.: Bact. Rev. **5**:181, 1941.

EXPLANATION OF FIGURE 4

A, *Spirochaeta pallida* (Noguchi strain). The specimen was from a three day culture on cysteine-ascitic fluid broth medium and was received from Dr. C. C. Kast. 60 kilovolts. Magnification as reproduced $\times 15,000$.

B, *Lactobacillus acidophilus*. The strain was recently isolated from saliva in a case of dental caries. The specimen was from a twenty-four hour culture on tomato agar. 60 kilovolts. Magnification as reproduced $\times 14,000$.

C, *Lactobacillus acidophilus*. The specimen was from a twenty-four hour culture on tomato agar. 55 kilovolts. Magnification as reproduced $\times 14,500$.

REGENERATION OF RAT LIVER AT DIFFERENT AGES

METABOLISM OF EMBRYONIC, NEONATAL AND
REGENERATING RAT LIVER

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Investigators have shown that liver tissue has a marked capacity to regenerate after partial hepatectomy.¹ With this fact established, it became desirable to determine (*a*) whether the age of the animal has any effect on the capacity or the rate of regeneration of rat liver after 65 per cent hepatectomy.

Beginning with the original work of Warburg,² a great deal of research has been done showing that neoplastic tissues in general are characterized metabolically by a high glycolytic activity of greater or lesser degree (despite one or two exceptions demonstrated by Murphy and Hawkins³ and others). Since regenerating liver is an exceedingly rapidly growing tissue, having a growth rate comparable to that of neoplastic tissue, it seemed profitable to determine by the Warburg technic (*b*) the metabolic activity of the regenerating liver and to compare it with that of malignant tissue.

Warburg and succeeding observers^{3a} have also established that embryonic tissues in general are characterized by a high anaerobic glycolytic activity. Because the rapid growth rate of regenerating liver is also comparable to that of embryonic tissues, it was decided to determine (*c*) the metabolic activity of embryonic and neonatal rat liver and to compare it with that of regenerating liver and of neoplastic tissues.

From the departments of pathology and biochemistry, Cornell University Medical College.

1. Fishback, F. C.: Arch. Path. **7**:955, 1929. Higgins, G. M., and Anderson, R. M.: *ibid.* **12**:186, 1931. Brues, A. M.; Drury, D. R., and Brues, M. C.: *ibid.* **22**:658, 1936.

2. Warburg, O.: Ueber den Stoffwechsel des Tumoren, Berlin, Julius Springer, 1926; cited by Barker and Summerson.⁴

3. Murphy, J. B., and Hawkins, J. A.: J. Gen. Physiol. **8**:115, 1925.

3a. Tamiya, C.: Biochem. Ztschr. **189**:175, 1927.

MATERIALS AND METHODS

Regeneration of Rat Liver at Different Ages After Partial (65 per Cent) Hepatectomy.—A total of 92 albino rats was used. The rats were arbitrarily divided into a young (0-100 Gm.), an intermediate (100-250 Gm.) and an old (250 Gm. and over) group. The partial hepatectomy consisted of resection of the left and median lobes (after ligation of their pedicles). The operations were performed with the rats under anesthesia induced with soluble pentobarbital. The resected tissue was weighed and pieces fixed for sectioning.

It was necessary to obtain a foundation from which to determine the weight of the liver tissue left in situ after removal of the left and median lobes of the

TABLE 1.—*Weight of Resected Left and Median Lobes as Percentage of Whole Liver Weight*

Rat Weight Group, Gm.	Weight of Left and Median Lobes Resected, Gm.	Weight of Residual Liver, Gm.	Total Weight of Liver, Gm.	Left and Median Lobes Resected as Percentage of Total Liver Weight
0-100.....	2.3	1.2	3.5	65
100-250.....	4.8	2.2	7.0	68
	5.2	1.2	6.4	81
	6.5	3.3	9.8	66
	4.2	2.2	6.4	65
	9.8	6.5	16.3	60
	5.5	5.2	10.7	51
	6.0	4.6	10.6	56
	6.2	2.7	8.9	69
	7.2	3.0	10.2	70
	8.3	4.3	12.6	65
	5.9	3.1	9.0	65
Average.....				68
250 and over....	5.5	3.1	8.6	63
	7.0	3.0	10.0	70
	6.4	5.1	13.5	55
	6.5	4.2	10.7	60
	8.1	3.1	11.2	72
	7.5	4.6	12.1	61
	4.9	2.2	7.1	68
Average.....				63
Grand average.....				65

liver, and from this in turn, to determine any increase in weight of the liver stimulated to regeneration by the partial hepatectomy. With this purpose in view, a number of rats were killed immediately after removal of the left and median lobes in the usual manner, and the weight of the remaining lobes was determined as well as that of the two resected lobes. The results of this procedure are shown in table 1. By averaging the results in each of the three age groups it is found that the weight of the resected left and median lobes for the younger age group was 65 per cent of the total liver weight, that for the second (intermediate) age group 68 per cent and that for the third (old) age group 63 per cent. For calculations of the percentage of regeneration, the weight of the left and median lobes removed at operation was considered to be 65 per cent of the weight of the original whole liver.

The liver was allowed to regenerate for periods up to twenty-one days after the partial hepatectomy, the animals being killed after a desired number of days. The residual liver was removed and weighed in order to determine any increase in

its weight since operation. Pieces of this tissue were fixed for sectioning, and in many cases fresh pieces were used for metabolic study.

Metabolism of Embryonic, Neonatal and Regenerating Rat Liver.—Each experiment was set up to determine the anaerobic glycolytic activity ($Q_A^{N_2}$) of the rat livers, and most of the experiments were also set up to determine the aerobic glycolytic activity ($Q_A^{O_2}$), oxygen consumption (Q_{O_2}), carbon dioxide elimination (Q_{CO_2}) and respiratory quotient (R. Q.) of the same livers.

$Q_A^{N_2}$ = cubic millimeters of carbon dioxide liberated per hour per milligram of dry weight of tissue by the action of lactic acid (produced by glycolysis of the dextrose in the medium) on the bicarbonate of the medium under anaerobic conditions.

$Q_A^{O_2}$ = cubic millimeters of carbon dioxide per hour per milligram of dry weight of tissue under aerobic conditions.

Q = cubic millimeters of carbon dioxide eliminated per hour per milligram of dry weight of tissue under aerobic conditions.

Q_{O_2} = cubic millimeters of diatomic oxygen consumed per hour per milligram of dry weight of tissue.

The aerobic determinations were made by using the Summerson differential manometer and Dixon-Keilin single side-arm aerobic vessels with potassium hydroxide well located in the stopcock underneath.

The anaerobic determinations were made by using the simple Warburg manometer and the Warburg single or double side-arm vessels.

The medium used was made up by mixing 100 cc. of Ringer's solution as modified by Warburg and Okamoto (1,000 parts of 0.9 per cent sodium chloride, 20 parts of 1.15 per cent potassium chloride, 20 parts of 1.22 per cent calcium chloride), 20 cc. of fifteen hundredth-molar sodium bicarbonate and 2.4 cc. of 10 per cent dextrose. A mixture of 95 per cent oxygen (O_2) and 5 per cent carbon dioxide was bubbled through the medium for thirty minutes just prior to the admission of the latter into the vessels. One or 2 cc. of the medium was placed in the anaerobic and 2 cc. in the aerobic vessels immediately before the tissue slices were placed in the vessels.

The animals were killed by a sharp blow to the base of the skull. In the case of fetuses, the mother was killed in the same manner, and the embryos were immediately removed. The livers were immediately removed and placed in Ringer's solution. Slices of the liver tissue (no thicker than 0.4 mm.) were cut freehand with a single-edged razor blade. The liver slices were blotted, weighed on a microtorsion balance and placed in the medium in the vessels. In all cases a representative sample of the slices of each liver was likewise blotted and weighed but then placed on a watch glass and dried over night in an oven at 60 C. The weight of this desiccated tissue was then taken and the percentage of the dry weight of that particular liver calculated.

For the anaerobic determinations, the manometers were now attached to a manifold and the system filled and equilibrated with a mixture of 95 per cent nitrogen (N_2) and 5 per cent carbon dioxide under a pressure of about 50 mm. of Brodie solution^{3b} for fifteen minutes, with an occasional shaking. The vessels were closed, the manometers placed on the bath and adjusted with shaking for five minutes. Readings were then taken for four hours, with shaking.

The temperature of the bath in all cases was 38 C. \pm 0.1.

3b. Dixon, M.: *Manometric Methods*, New York, Cambridge University Press, 1934, p. 9.

For the aerobic determinations (with which we are less concerned in this paper), the tissue-containing vessels were placed on their manometers and the manometers in turn placed on the bath, where they were subjected to a mixture of 95 per cent diatomic oxygen and 5 per cent carbon dioxide under pressure of about 50 mm. of Brodie solution, with shaking, for fifteen minutes. The vessel system was then equilibrated to atmospheric pressure for one or two minutes.

In a number of experiments, the lactic acid produced by the liver tissue during the course of the experiment was analyzed quantitatively by the chemical method devised by Barker and Summerson.⁴

The exact procedure used in these experiments, together with a discussion of the theory and calculations involved therein, is given in full by Burk and co-workers.⁵

RESULTS

Regeneration of Rat Liver at Different Ages.—The individual weight changes for the three age groups are presented graphically in figure 1 *A* in order to show the variations within the groups. In figure 1 *B* are shown curves based on the average values for the three groups. The weight of residual liver, expressed in percentage of original whole liver, is plotted as a function of time for regeneration after operation in days.

It is seen in figure 1 *B* that for the first two days after partial hepatectomy the curves for all three rat age groups rise rapidly in a linear fashion and at approximately the same rate. At this time the weight of residual liver has reached 65 per cent of the original whole liver weight (having started at 35 per cent by operation). After this, the curve for each age group proceeds as follows:

The curve for the young group rises more sharply than the others in a linear fashion, reaching a peak of about 145 per cent after one week and then falling off to about 110 per cent at the end of fifteen days.

The intermediate age group shows a curve which rises more slowly, reaching about 120 per cent at eight days and then leveling off thereafter.

The curve for the older group rises considerably more slowly after the second day, gradually reaching an apparent climax of 95 per cent at about the ninth day.

Morphology of the Regenerating Tissue.—Grossly, the enlargement of all lobes seemed to be uniform. For the first two days after operation, the liver tissue was yellow and friable, with the consistency of fresh brain. Thereafter, the tissue assumed the reddish brown color of normal liver and became more firm in consistency. By the fifth day after operation the residual lobes had the appearance of normal liver except that the lobules were larger and more prominent than usual.

4. Barker, S. B., and Summerson, W. H.: J. Biol. Chem. **138**:535, 1941.

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Microscopically, the regenerating livers showed marked changes, which were uniform in each of the age groups. During the first two days the cells appeared slightly larger than normal. No changes were seen in the nuclei. The cytoplasm was increased slightly in amount and contained small vacuoles and irregular clear spaces. The cytoplasm appeared to be condensed at the periphery of the cells, and cell boundaries, while not thickened, appeared more prominent and refractile.

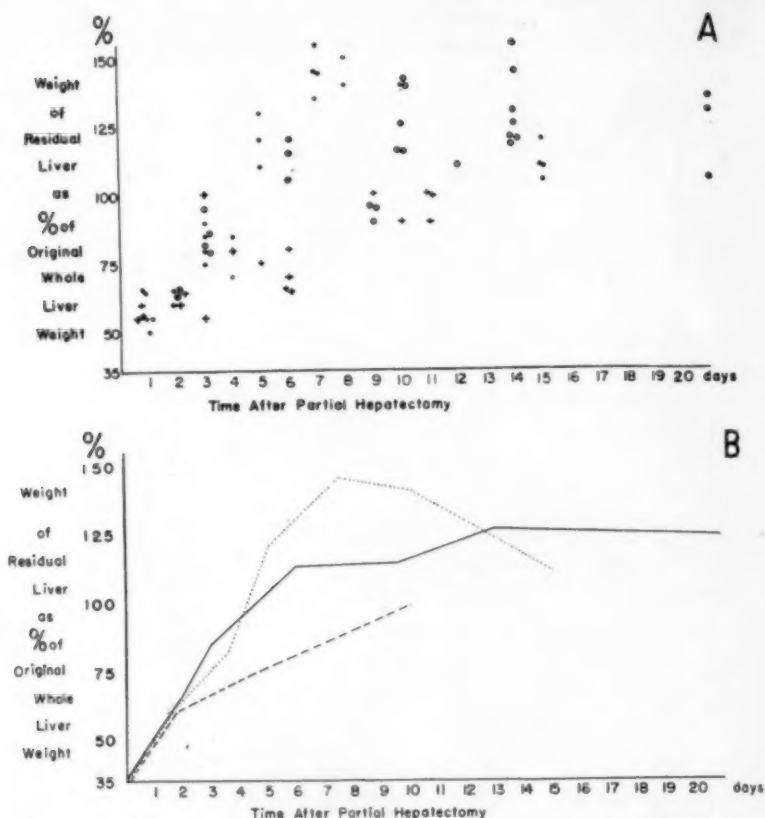


Fig. 1.—*A*, scatter diagram showing the percental regeneration of liver in relation to days following partial (65 per cent) hepatectomy in young rats (dots), in old rats (crosses) and in middle-aged rats (circles). *B*, curves of the rate of regeneration of liver based on averaged values of *A* in young (dotted line), old (interrupted) and middle-aged rats (continuous line).

On the second day this same process was more marked, and, in addition, an occasional mitotic figure was seen. Mitoses were most abundant in the livers on the third and fourth days of regeneration, as many as four mitotic figures being seen in fields under high dry magnification, and the already described changes in the cytoplasm were more advanced.

Mitotic figures were less frequent in livers after the fourth day of regeneration, and at twenty-one days the liver tissue appeared normal microscopically except that the lobules were conspicuously large.

Metabolism of Rat Liver Regenerating after 65 per Cent Hepatectomy.—The results of the metabolic studies are summarized in table 2. The representative normal control livers show a slightly higher rate of anaerobic glycolysis for the liver of a 21 day old rat ($Q_A^{N_2}$ 0.8) than for an actually senile 2 year old rat ($Q_A^{N_2}$ 0.5), with a 1½ year old rat intermediate at 0.6.

TABLE 2.—Metabolism of Rat Liver Regenerating After 65 per Cent Hepatectomy

Rat Weight, Gm.	Regeneration		$Q_A^{N_2}$	$Q_A^{O_2}$	Q_{CO_2}	Q_{O_2}	R. Q.
	Percentage	Days					
392	54	1	1.0	1.2	0.4	1.1	0.42
320	66	1	0.7	0.6	1.4	1.7	0.79
361	62	2	0.6	0.8	1.7	4.5	0.38
318	65	2	0.7	1.1			
367	54	3	0.5				
349	54	3	0.1	0.6	1.8	4.2	0.44
316	100	3	0.5	0.6	1.8	3.0	0.57
306	62	5	0.1	0.6	
308	66	6	0.6				
336	65	6	0.6				
296	101	9	0.6	1.7	3.1	3.5	0.72
300	100	11	0.7	0.5	3.8	5.2	0.72
205	115	10	0.6	0.7	1.6	2.3	0.67
188	131	10	0.6	0.8			
80	58	1	0.6	0.9	0.4	0.9	0.39
76	58	1	0.7	(-0.5)	3.2	3.6	0.82
52	80	3	2.1				
51	75	3	1.6				
34	100	3	1.8	1.5	5.7	3.5	0.60
22	90	3	0.8				
52	70	4	1.5				
100	110	5	0.8				
100	121	5	0.5				
95	135	7	0.5				
Representative normal livers							
90	Normal	21 days	0.8	1.7	3.0	5.7	0.52
340	Normal	1.5 yr.	0.6	1.5	0.5	1.4	0.37
210	(Normal) Senile	2.0 yr.	0.5	1.3	1.5	3.3	0.46

The regenerating livers of rats weighing over 50 Gm. show that the amount of anaerobic glycolysis is of the order of $Q_A^{N_2}$ 0.5 to 0.6 in most instances, with extremes of 0.1 and 1.0. The rate of anaerobic glycolysis of regenerating liver for rats weighing over 50 Gm. was found not to vary with the age of the animal, length of time after partial hepatectomy or percental increase in weight of residual liver.

The values of $Q_A^{N_2}$ for regenerating liver of rats weighing less than 50 Gm. were found to be slightly higher, varying from 0.8 to 2.1. This is, however, within the range found by Hawkins⁶ for the normal liver of rats of this weight.

6. Hawkins, J. A.: J. Gen. Physiol. **11**:645, 1928.

Under aerobic conditions no correlation was found between values of aerobic glycolysis ($Q_A^{O_2}$), oxygen consumption (Q_{O_2}) or respiratory quotient (R.Q.) and the experimental variables, such as age of the animal, amount of regeneration, and time after partial hepatectomy.

Metabolism of Embryonic and Neonatal Rat Liver.—The results are summarized in table 3. It is seen that the rate of anaerobic glycolysis is very high for the youngest embryonic liver studied ($Q_A^{N_2}$ 8.9), the weight of which was only 60 mg. This rate decreases slightly but remains high until birth. After birth, however, there is a rapid and marked fall in the rate of anaerobic glycolysis of the liver to $Q_A^{N_2}$ 6.4 at eight hours and then 2.7 at twenty-four hours after birth. From this point on there is a much more gradual decrease to $Q_A^{N_2}$ 1.0 at nine days.

TABLE 3.—*Metabolism of Embryonic and Neonatal Rat Liver*

No.	Animal		Liver		$Q_A^{N_2}$	$Q_A^{O_2}$	Q_{CO_2}	Q_{O_2}	R. Q.
	Age	Weight, Gm.	Weight, Mg.	Per-centage of Body Weight					
3	Fetal	0.80	60	7.4	19.0	8.9+			
4	Fetal	1.09	93	8.1	21.0	10.0+			
9	Fetal	1.26	110	8.7	19.3	8.1	0.6	6.0	1.0
10	Fetal	2.12	185	8.7	19.8	8.0	0.6	6.7	1.0
2	8 hr.	4.6	240	5.3	20.3	6.4			
1	1 day	4.7	186	3.7	2.7			
11	1 day	5.5	215	3.9	20.0	2.3	1.2	4.4	0.66
14	2 days	7.5	336	4.5	21.0	2.2			
15	3 days	5.4	262	4.8	24.4	1.7			
13	9 days	14.0	350	2.5	19.6	1.0			
8	21 days	30.0	1,052	3.5	17.9	0.8	1.7	3.0	0.52
7	18 mo.	340.0	8,830	2.6	20.2	0.6	1.5	0.5	1.4
6	24 mo.	210.0	5,815	2.8	17.5	0.5	1.3	1.5	3.3
									0.46

When the microscopic appearance of these embryonic and neonatal livers (as shown in figure 2) is compared with their rates of anaerobic glycolysis, it is seen that the rate of anaerobic glycolysis of a particular liver is closely paralleled by the number of erythropoietic cells in that liver. Thus in table 3 rat 3, a fetal rat weighing 0.8 Gm., has the highest $Q_A^{N_2}$, 8.9, and at the same time the greatest number of erythropoietic and myelogenic cells and megakaryocytes per field, as shown in A of figure 2, while rat 15, a 3 day old rat weighing 5.4 Gm., has a low $Q_A^{N_2}$ of 1.7 and at the same time a small number of early blood cells (fig. 2 D).

It is of interest that the embryonic liver had a low value for aerobic glycolysis (0.6) and high values for oxygen consumption (approximately 6.0) and carbon dioxide liberation (approximately 6.0). An R. Q. of 1.0 is characteristic of embryonic tissue.

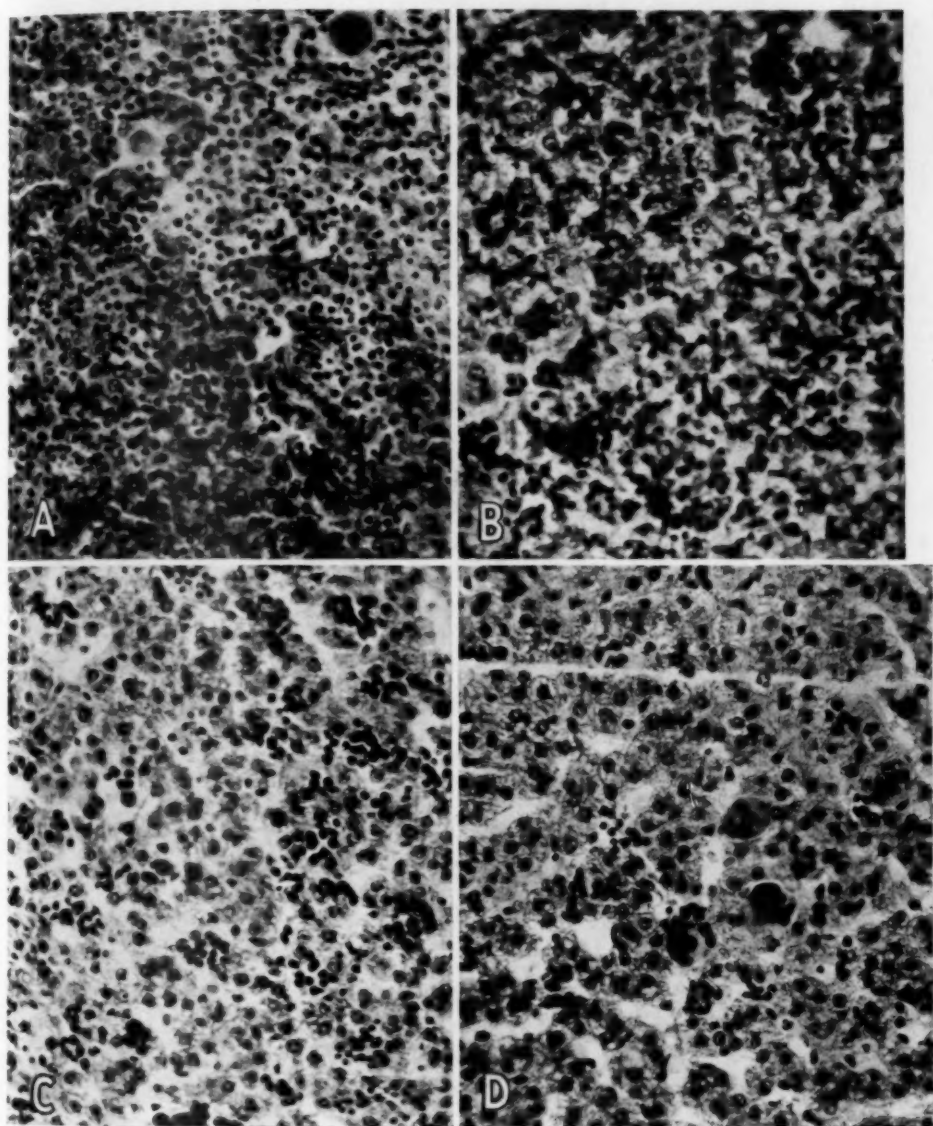


Fig. 2.—*A*, liver of fetal rat 3; weight, 0.8 Gm. $Q_A^{N_2} 8.9 +$. *B*, liver of fetal rat 10; weight, 2.12 Gm. $Q_A^{N_2} 8.0$. *C*, liver of a 2 day old, rat 14; weight, 7.5 Gm. $Q_A^{N_2} 2.2$. *D*, liver of a 3 day old, rat 15; weight, 5.4 Gm. $Q_A^{N_2} 1.7$.

COMMENT

Regeneration of Rat Liver at Different Ages.—Figure 1A shows that increase in liver weight after the initial two days of cellular swelling following partial hepatectomy is modified by the age of the animal, being greatest and most rapid for the young age group, while considerably less and considerably slower for the old age group, with the intermediate age group pursuing an intermediate course. This suggests that a young liver cell has the capacity to regenerate at a greater rate than has an older cell. It would appear then that hepatic damage (partial destruction of the liver) occurring in an old subject is thereby a more serious matter than when occurring in a younger one in that a longer period might be required for repair.

Metabolism of Rat Liver Regenerating after Sixty-Five per Cent Hepatectomy.—The results of this part of the experiment show that the anaerobic glycolysis of the regenerating liver was increased above the value for normal adult liver only in the case of young animals weighing 50 Gm. or less, and, according to figures obtained by Hawkins,⁶ this degree of glycolysis is within normal limits for rats of that age.

No correlation can be found between $Q_{\text{A}}^{\text{N}_2}$ value and length of time for regeneration or amount of regeneration.

From the fact that this actively regenerating rat liver (an extremely rapidly growing tissue, having a growth rate of embryonic or neoplastic dimensions) has an anaerobic glycolytic activity no greater than that of the resting liver of the normal rat, the following conclusion has been arrived at: that the anaerobic glycolytic activity of a tissue is not dependent on the growth rate or degree of general activity of the tissue but is an inherent property of the tissue and characteristic for that particular tissue.

Metabolism of Embryonic and Neonatal Rat Liver.—The heightened anaerobic glycolysis of the liver's embryonic life would at first appear to be due to the activity of growth of the embryonic liver.

It has been shown in an earlier paragraph, however, that there is a close parallelism between the degree of erythropoietic involvement and the degree of anaerobic glycolytic activity of the embryonic and the neonatal liver.

Also, it has been found by Sprince, Kabat, Furth and Burk,⁷ using chicken bone marrow, and by Warren,⁸ using rabbit bone marrow, that the erythropoietic fraction of bone marrow has a $Q_{\text{A}}^{\text{N}_2}$ value of about 7 to 8, while Warren also found that the myelogenic fraction of rabbit bone marrow has a $Q_{\text{A}}^{\text{N}_2}$ value of 21 and the normal whole bone marrow a value of 14.

7. Sprince, H.; Kabat, E.; Furth, J., and Burk, D.: To be published.

8. Warren, C. O.: Am. J. Physiol. **131**:176, 1940.

Therefore, it appears possible and indeed highly probable that the high anaerobic glycolytic activity of embryonic rat liver is due to the metabolism of the hematopoietic system within the structure of the embryonic liver and not due, as previously thought, to the activity of the growing and multiplying liver cells themselves. While because of the great difficulty of removing the hemopoietic cells from the embryonic liver it is not possible to prove that the high rate of glycolysis is due to the hemopoietic cells, it is for the same reason impossible to disprove that such is the case.

SUMMARY

Regeneration of liver following partial (65 per cent) hepatectomy was compared in rats of different ages, and the metabolism (aerobic and anaerobic glycolysis, oxygen consumption and carbon dioxide production) of embryonic, neonatal and regenerating rat liver was studied.

Rat livers at all ages studied had a great capacity to regenerate after partial hepatectomy, but the rate and the total amount of regeneration varied inversely with the age of the animal.

The rate of anaerobic glycolysis of regenerating liver was not increased over that of the normal resting liver in rats of comparable age and was independent of the length of time following partial hepatectomy and of the total amount of regeneration.

The rate of anaerobic glycolysis of embryonal rat liver was conspicuously higher than that of neonatal liver, and the rate for neonatal liver was in turn increased over that of normal adult liver. The high rates during the embryonal and neonatal periods could be correlated directly with the amounts of hemopoietic tissue in the liver at these periods.

The increase in anaerobic glycolysis of neoplastic tissue over that of non-neoplastic tissue is not due to an increase in growth rate, but rather to differences inherent in and characteristic of the two types of tissue.

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STUDIES OF SPONTANEOUS TUMORS IN GUINEA PIGS

II. TUMORS OF THE STOMACH AND INTESTINE

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AND

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During the last twenty years one of us (G. N. P.) has collected about 100 spontaneous tumors that were found in autopsies of approximately 7,000 guinea pigs, which had been used for various experimental studies. The relatively high percentage of tumors is explained by the fact that this colony of guinea pigs included many senile animals. Tumors are rarely found in guinea pigs that are less than 4 or 5 years old. The material was originally collected in connection with the more general study of the problem of senility. It was soon realized, however, that spontaneous tumors in senile guinea pigs were more frequent than had been anticipated, and that their study should be treated as an independent problem.

Eight of the collected tumors were found attached to the stomach or the intestine. One of these, a fibromyoma of the stomach, associated with an adenoma of the right adrenal, has been described.¹ The others are presented in this paper. Considering that tumors of the stomach and intestine were not particularly rare in this stock, one finds it difficult to explain the absence of previous descriptions of similar tumors by other investigators.

This presentation is limited chiefly to the morphologic characteristics of the tumors. More detailed biologic data and observations on the endocrine glands and other organs will be given later.

TUMORS ATTACHED TO THE STOMACH

ANIMAL 1.—A female guinea pig (4754) that had been subjected to over-feeding for several years died when 6 years old.

The ovaries were enlarged and cystic. There was a tumor, 0.75 cm. across, protruding from the external wall of the lower part of the uterus at the level of the cervix. There was also a tumor, 5 by 4 by 2.7 cm. in size, protruding from the greater curvature of the stomach close to the pylorus. The point of attachment

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1. Papanicolaou, G. N., and Olcott, C. T.: *Am. J. Cancer* **40**:310, 1940.

measured 1.4 by 0.7 cm. The tumor was firm but not hard. A piece of this tumor was transplanted into guinea pig 4641, in which a subcutaneous sarcoma developed some distance from the site of inoculation. This was probably a spontaneous tumor. It will be described later.

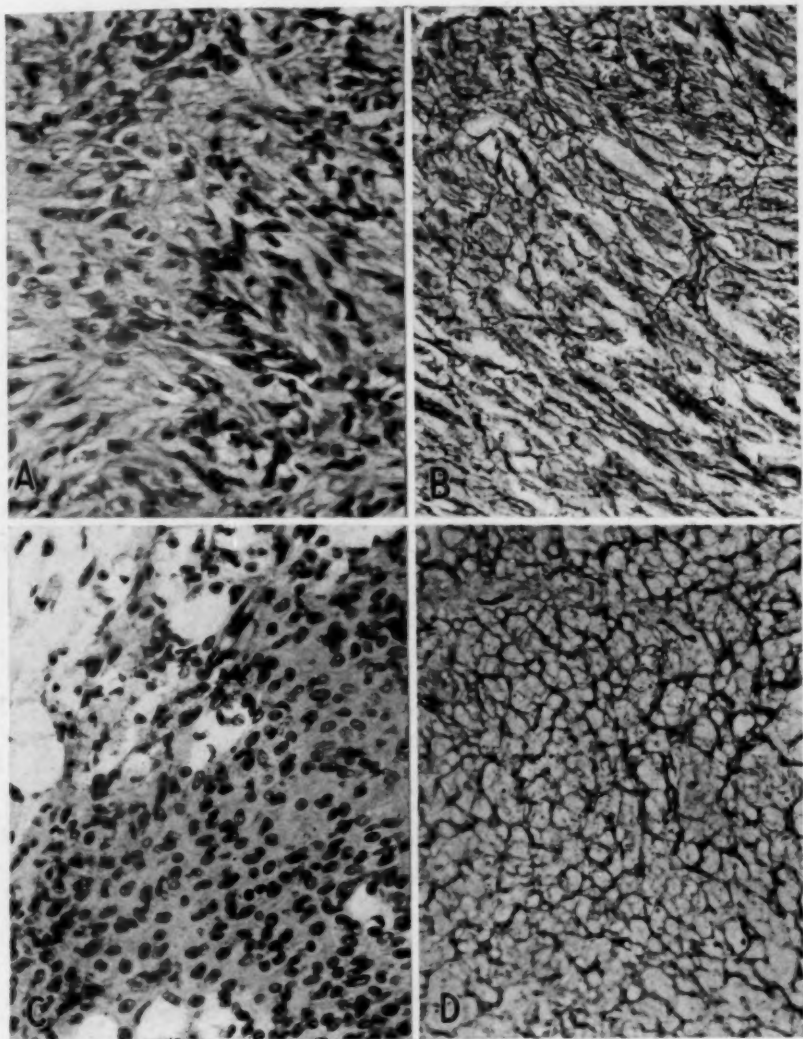


Fig. 1.—*A*, section of the leiomyoma attached to the stomach of animal 1. Modified Masson's stain; $\times 255$. *B*, same as *A* stained with Foot and Foot's silver stain, showing fibers of reticulum between the individual cells $\times 255$. *C*, liposarcoma attached to the intestine of animal 5. Modified Masson's stain; $\times 255$. *D*, same as *C* stained with Foot and Foot's silver stain, showing distribution of fibers of reticulum around single cells. $\times 255$.

Microscopic Examination.—Material from the tumor attached to the stomach fixed in Bouin's solution was examined. The prevailing tissue was smooth muscle, which was arranged in nodules and formed whorls separated from one another by thin areas of connective tissue. The muscle fibers were spindle shaped and ran in all directions. They were loosely arranged and were separated by small irregular clefts (fig. 1 A). The nuclei of the muscle fibers showed some variability in size. Strands of connective tissue ran through the tumor and contained dilated arteries and many dilated veins. Extravasations of serum, red blood cells and fibrin were also found. Around the areas of extravasation were occasional groups of lymphocytes and histiocytes. Some of the latter were loaded with blood pigment. In frozen sections there were only very small foci of fat. Study of the point of attachment showed that the tumor was adjacent to the inner circular and oblique layer of muscle of the stomach. It was clearly separated from the muscularis mucosae.

Material was studied after fixation in solution of formaldehyde U. S. P. and treatment with Zenker's solution. One specimen stained with Foot and Foot² silver stain revealed a clear network of fine reticulum fibers extending between the individual cells as in normal smooth muscle (fig. 1 B).

Diagnosis: Leiomyoma attached to the stomach.

ANIMAL 2.—A female guinea pig (6018) was born June 8, 1934. There was an abscess of the right mamma in February 1938. She had a litter May 7 and died of pneumonia ten days later.

At autopsy the left ovary was totally cystic and the right contained small cysts. There was a tumor attached to the external wall of the greater curvature of the stomach, 2 cm. from the pyloric end. At the point of its attachment it slightly protruded into the cavity of the stomach, where it formed a small crater in the mucosa. The tumor was rather soft and white. Part was fixed in Zenker-formaldehyde solution,³ and part was used for transplantation into other guinea pigs. Transplantation gave uniformly negative results.

Microscopic Examination.—The general structure was similar to that of the tumor of animal 1 (4754). At the crater the gastric mucosa showed chronic inflammatory changes with very numerous plasma cells. Plasma cells and histiocytes extended into the adjacent tumor tissue. Material stained by the Foot and Foot² stain showed the arrangement of reticulum characteristic of myoma. It was similar to that already shown in figure 1 B.

Diagnosis: Leiomyoma attached to the stomach; chronic inflammation of the adjacent gastric mucosa.

ANIMAL 3.—A male guinea pig (4218) approximately 4 years old died with pneumonia. Autopsy disclosed no abnormalities except for a tumor, 1.7 by 1 cm. in size, attached to the greater curvature of the stomach.

A microscopic examination was made of material fixed in Bouin's solution. The general structure of the tumor resembled that of the tumors described in animals 1 and 2.

In the part of the tumor farthest removed from the point of attachment there was an ovoid area of degenerated muscle tissue, measuring 0.5 by 0.3 cm. The cytoplasm of the cells had become amorphous and the nuclei pyknotic. Between

2. Foot, N. C., and Foot, E. B.: Am. J. Path. 8:245, 1932.

3. This is Zenker's solution prepared with solution of formaldehyde U. S. P. instead of acetic acid.

the network formed by the cells there were hollow spaces. The degenerating mass was surrounded by connective tissue, which gave off branches that penetrated between the tumor cells. The tissue was edematous and was infiltrated by hemolyzed blood. Histiocytes were present in large numbers. Many of them engulfed blood pigment. Dilated blood vessels were conspicuous in the connective tissue at the periphery of the tumor. Blood vessels were few between the muscle cells.

Diagnosis: Leiomyoma attached to the stomach, showing focal degeneration.

ANIMAL 4.—A male guinea pig (5697) died during hot weather at the age of approximately 6 years. The animal had been bought from a dealer. There were no significant findings at autopsy except a tumor, 3 by 1.5 by 1 cm. in size, attached by a pedicle 0.5 cm. long to the greater curvature of the stomach approximately 1.7 cm. from its pyloric end. The tumor was flattened and was brownish yellow, resembling fat. It was fixed in Bouin's solution.

Microscopic Examination.—The bulk of the specimen consisted of fat. Most of the cells were necrotic, so that a satisfactory staining reaction was not obtained, but many cells could be readily identified as adipose cells. There were many blood vessels, which were poorly outlined, and many dilated capillaries. Thin strands of connective tissue were found under the mesothelium and around the large blood vessels. In certain places groups of spindle-shaped fibers were seen, but their positive identification was impossible, owing to their advanced degeneration. Large numbers of histiocytes occurred singly and in small nests, especially adjacent to degenerated blood vessels. Some cells were very large and evidently represented giant cells of histiocytic origin. Tissue stained after frozen sectioning was found to consist largely of fat.

Diagnosis: Lipoma attached to the stomach, showing advanced degeneration.

TUMORS ATTACHED TO THE INTESTINE

ANIMAL 5.—A guinea pig (5662) was bought from a dealer in the summer of 1928. Dec. 14, 1932, when the animal was pregnant, both ovaries were removed. The left ovary was cystic, and the right showed early cystic degeneration. Thirteen days after the operation, the guinea pig delivered three dead premature young. Starting March 20, 1933, she received three injections of a corpus luteum extract. May 29, 1934, a tumor was noted by palpation of the abdomen, evidently attached to the intestine. June 20, the animal was operated on. There was a tumor about 2.5 by 1.5 cm. in size attached to the small intestine (fig. 2A). The point of attachment was 1.0 cm. long. The tumor was soft and vascular. There were many blood vessels outlined on its surface. A piece of tissue was removed as indicated by the shaded area and transplanted to 4 animals with negative results. The animal was in good condition after the operation. Biopsy showed a structure similar to that described for the autopsy specimen.

November 1, one hundred and thirty-four days later, the animal became weaker and was killed. The tumor had grown considerably since the operation and was now 3.5 cm. long (fig. 2B). It had increased in width and thickness, more particularly on the end corresponding to the site of removal, where it was 2.0 cm. wide. There were adhesions to the large intestine and the surrounding tissue that were not present at the time of operation. The tumor was vascular, soft and brittle, but there were no metastatic nodules in the peritoneum. The left lung was hepatized and adherent to the thoracic wall. At the point of adhesion

of the lung to the wall there was a well defined mass of firm tissue. The right lung and the heart were normal. The thyroid was enlarged and rounded. There was a dark blood spot over the posterior part and of the right lobe.

Microscopic Examination.—The bulk of the tumor consisted of a compact mass of ovoid cells showing very slight variability in size and form (fig. 1 C). The outline of these cells was ill defined. The cytoplasm showed no definite structure and stained lightly. Some of the cells contained fine vacuoles. The nuclei were ovoid, and the chromatin was finely granular. There were some mitotic figures. Between the cells, fine fibrils were visible, which formed a reticulated structure and stained green with Masson's ⁴ technic. There were no large collagenous fibers.

In certain places the fat cells had more abundant cytoplasm containing many fine vacuoles. These cells resembled fat cells and showed transition forms to the cells forming the bulk of the tumor. The tumor was not unusually vascular. It contained small capillaries in moderate numbers, but only a few larger vessels. Scattered between the cells were vacuoles of varied size. These were lined by elongated cells with scant cytoplasm. They evidently represented fat tissue, in which the fat had been dissolved out in the preparation of paraffin sections. The

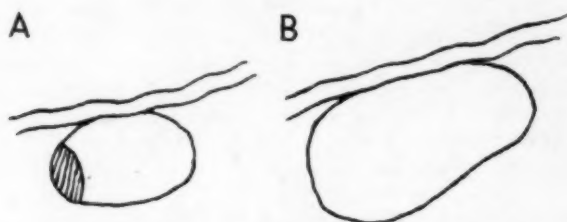


Fig. 2.—Outlines of the tumor attached to the small intestine of animal 5: *A*, as it appeared during operation, the shaded area showing the part removed at operation; *B*, as it appeared at autopsy (natural size).

vacuoles were more numerous toward the periphery of the tumor, adjacent to the serosa, and were less frequently found in the part of the tumor adjacent to the intestine.

At the point of attachment the tumor was separated from the intestinal wall by a layer of normal fat. There was no inflammatory reaction in the muscular and mucous coats, which were normal in appearance.

A section of this tumor stained by the Foot and Foot silver technic (fig. 1 D) showed innumerable small round areas, each surrounded by definite circular fibers of reticulum.

Diagnosis: Liposarcoma attached to the intestine.

The firm tissue adjacent to the left lung had no definite line of demarcation. On microscopic examination it was a fibrous structure, consisting chiefly of cells resembling fibroblasts. These cells were separated by small irregular spaces, in which there was a slight infiltration of leukocytes. There were a few small nodules of shorter, thicker and more compactly arranged cells. These cells somewhat resembled, but could not be clearly identified as of the same type as, those found in the abdominal tumor. There were many small vessels in the

4. Masson, P.: *J. Tech. Methods* 12:75, 1929.

tumor, and in some of them the endothelial lining was slightly thickened. Considered by itself, the thoracic tumor would be considered as fibrosarcoma. It is, however, possible that it represented a reaction to a metastasis from the abdominal tumor.

Diagnosis: Fibrosarcoma (?) of the left lung adhering to the thoracic wall,

ANIMAL 6.—A female guinea pig (5652) was bought in the same lot as animal 5 in the summer of 1928. In October 1933, the animal received seven subcutaneous injections of an extract of beef adrenal cortex in corn oil. In May 1934 a tumor was palpated in the abdominal cavity. The animal died June 10 when approximately 6 years old.

There was a tumor 2.0 by 1.5 cm. in size attached to the wall of the lower part of the small intestine. It was soft and brittle with many dark bloody areas. The tumor was transplanted, with negative results. A smaller tumor, pale, harder and completely detached, was present adjacent to the pyloric end of the stomach.

Microscopic Examination.—Several sections of the tumor attached to the intestine showed it to consist of elongated cells running in various directions and forming irregular whorls. The cells were poorly outlined and were separated by small irregular clefts. The cellular arrangement showed a superficial resemblance to the tumors already described as leiomyoma. On closer examination, however, the cells showed a palisade-like arrangement, suggesting Verocay bodies.⁵ The cells were smaller and had smaller and denser nuclei than cells of smooth muscle origin. In sections stained with the Foot and Foot silver stain the fibers of the reticulum enclosed groups of cells but did not run between individual cells. The appearance was clearly that of a tumor derived from a nerve sheath.

Diagnosis: Neurilemmoma attached to the intestine.

Microscopic examination of the mass adjacent to the pyloric end of the stomach showed the structure of leiomyoma as described in animals 1, 2 and 3. The silver stain showed a well developed reticulum running between the individual cells (fig. 1 B).

Diagnosis: Leiomyoma adjacent to the stomach.

ANIMAL 7.—A female guinea pig (4931) was born June 18, 1923. May 6, 1925, the left ovary was removed. Oct. 23, 1929, an intestinal tumor was palpated. After the palpation the animal appeared sick and was killed two days later, at the age of 6 years and 4 months.

At autopsy there was a nonpedunculated mass 2.5 by 1.8 cm. in size attached to the wall of the small intestine, 38 cm. above the cecum. There were some bloody areas on the surface of this mass. Fresh blood and fragments of tissue were present in the peritoneal cavity, indicating probable rupture of the large tumor during palpation. There were several nodules in the mesentery and other parts of the peritoneal cavity. The liver and spleen were normal. The right lobe of the thyroid was normal; the left was large, and there was a dark nodule in the tissue adjacent to its lower end. The right ovary was normal, but there was a small parovarian cyst at the site of the ablated left ovary. Pieces from the large tumor were transplanted to 5 animals, with negative results.

Microscopic Examination.—By study of the point of attachment of the tumor to the small intestine the growth was found to be located at the level of the circular layer of smooth muscle (fig. 3). At one point the circular muscle layer fused with the tumor (fig. 4 A). The longitudinal muscle was seen in some places and appeared to be normal (fig. 4 B). It lay external to the neoplasm.

5. Verocay, J.: Beitr. z. path. Anat. u. z. allg. Path. 48:1, 1910.

In these areas some ganglions of the plexus of Auerbach were seen between the longitudinal muscle and the underlying peripheral part of the tumor. These topographic relations indicate the existence of a close connection and a partial fusion between the tumor mass and the circular muscle layer.

The structure of the tumor was uniform in its central part (fig. 4 D). It consisted of elongated cells which had ovoid and deeply stained nuclei. The cell outlines were indistinct. Many of the cells were cut crosswise, and their nuclei were small and rounded, and formed dense groups. The general arrangement of the nuclei showed the palisade formation characteristic of Verocay bodies.⁴ In sections stained with silver the reticulum surrounded the cells in groups but not individually. On the basis of these findings the tumor should be interpreted as being of nerve sheath origin. In fact, one could see in several places a great proliferation of cells around the sheaths of nerves that penetrated the tumor (fig. 4 C) or around those of the ganglions of Auerbach's plexus. In the part of the tumor which fused with the circular muscle layer (fig. 4 A) the cells of

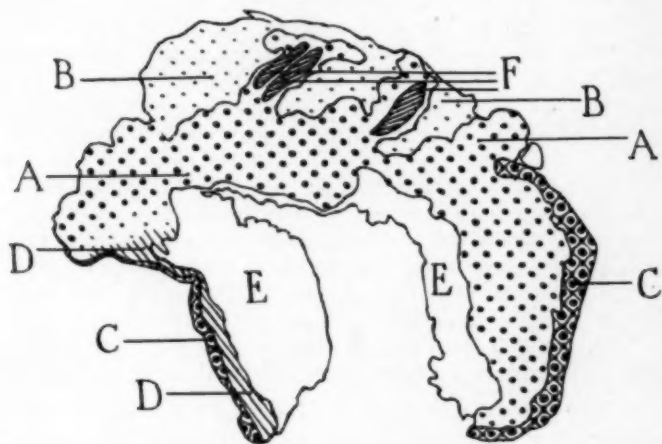


Fig. 3.—Drawing made from a microscopic slide of animal 7; $\times 11+$. It shows the relation of the tumor to the wall of the intestine. A indicates well preserved neurilemma; B, neurilemma showing degeneration; C, longitudinal muscle; D, circular muscle running into tumor (see fig. 4 A); E, mucosa; F, nerves. The sheaths of the latter are continuous with the tumor (fig. 4 C).

the tumor were closely interlaced with smooth muscle fibers. The longer and lighter stained nuclei of these fibers could be traced for some distance within the tumor. Similar interlacing of the two cell types occurred in the peripheral part of the tumor which came in contact with the inner margin of the longitudinal muscle (fig. 4 B). In a section of this region stained with silver (fig. 4 E) three distinct zones could be recognized, each one with a different arrangement of the reticulum fibers; an outer one of longitudinal muscle, a middle one in which circular muscle fibers prevailed and an inner one which showed the characteristic arrangement of the Verocay bodies.

The outer zone showed a normal structure and was distinctly separated from the underlying tissue. The fibers of reticulum penetrated between the individual cells. The structure of the middle zone was less typical, and there was no

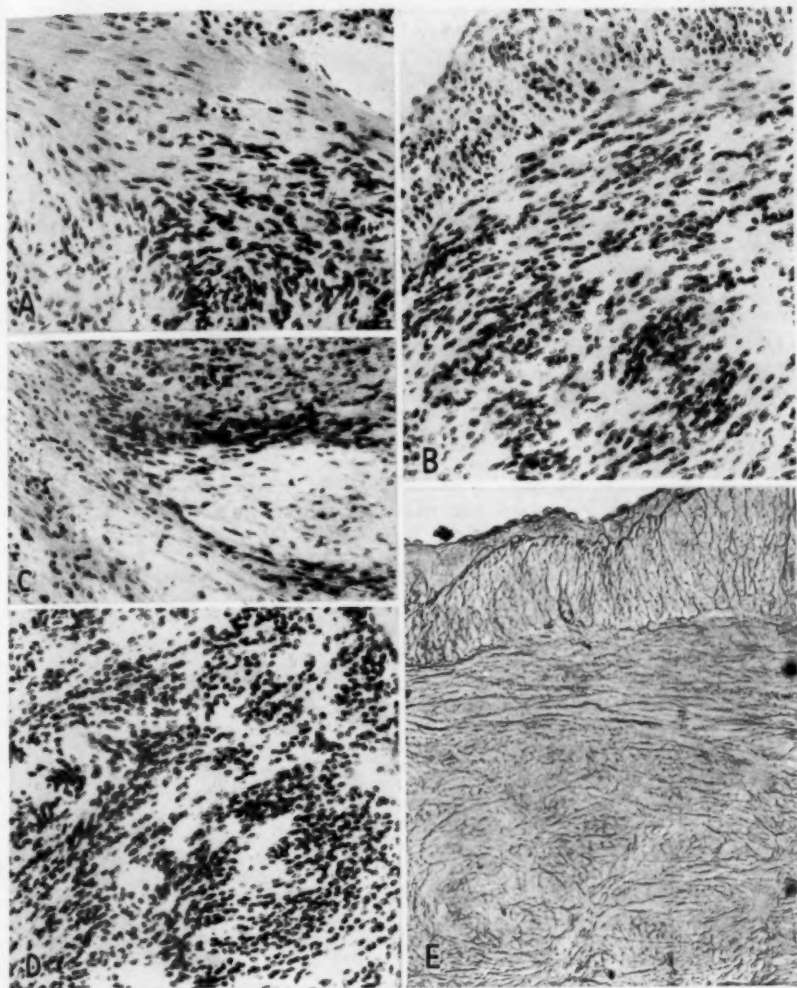


Fig. 4.—*A*, section of neurilemmoma attached to the intestine of animal 7, showing fusion of the circular layer of the intestinal muscle (at the upper left corner) with the tumor. Foot's modified Masson's stain; $\times 118$. *B*, peripheral part of the neurilemmoma of animal 7 in contact with normal overlying longitudinal muscle of the intestine. Adjacent to the longitudinal muscle, smooth muscle fibers of the circular layer predominate. Foot's modified Masson's stain; $\times 118$. *C*, section of the neurilemmoma of animal 7 showing extensive proliferation of the cells of the sheath of a nerve penetrating the tumor mass. Foot's modified Masson's stain; $\times 118$. *D*, central part of the neurilemmoma of animal 7, showing Verocay bodies. Foot's modified Masson's stain; $\times 118$. *E*, section of the peripheral part of the neurilemmoma of animal 7 (see *B*) stained with Foot and Foot's stain. The difference in distribution of reticulum fibrils in the normal longitudinal muscle, in the underlying peripheral part of the tumor and in the deeper part of the tumor is seen. $\times 118$.

sharp line of demarcation separating it from the inner zone. It appeared that here again the tumor merged with the zone of the circular muscle and that the respective cellular elements became intermixed near the point of fusion. There were areas in the tumor which showed degenerative changes. There were also places showing localized necrosis and extravasation of blood with many histiocytes.

Diagnosis: Neurilemmoma invaded by smooth muscle cells, attached to intestine.

A piece of mesentery containing a scarcely visible nodule was sectioned. The nodule showed a structure similar to the neurilemmomatous part of the tumor attached to the intestine. Several mitotic figures were present. Close to this nodule there were six similar microscopically visible foci, two of which were in the process of degeneration. All of these areas were round and appeared to be independent of one another.

Diagnosis: Multiple neurilemmoma of the mesentery.

The nodule adjacent to the thyroid was found to be adenomatous and will be described later with other tumors of the thyroid.

COMMENT

The tumors reported in this paper were grouped together because of their topographic relations to the stomach and intestine. Histologically, they were of four different types. Four of the five tumors related to the stomach were of myomatous nature and were classified as leiomyoma. Their structure was essentially similar to that of the tumor attached to the stomach of animal 5689, which was described as fibromyoma in a paper published elsewhere.¹ Only one of the tumors connected with the stomach was diagnosed as lipoma, that of animal 4; it showed extensive degeneration. Some of the other tumors also showed degenerative changes but of localized character.

One common characteristic of the tumors related to the stomach was that each developed on the side of the greater curvature and near the pyloric end. The myomatous tumors showed a close contact with the circular and oblique layers of the muscular coat.

The three tumors attached to the intestine were of different nature. They were all attached to the small intestine. Those in animals 6 and 7 were classified as neurilemmoma and that in animal 5 as liposarcoma. The classification as neurilemmoma was based on the morphologic characteristics of the cells and of their arrangement, as well as on the distribution of the reticulum, as demonstrated by the Foot and Foot silver technic.² The term "neurilemmoma" was coined by Stout⁶ and has advantages over other designations of the tumor, such as "lemmoma," "schwannoma," "peripheral glioma" and "fibroblastoma." The evidence presented by Stout,⁶ Foot⁷ and Murray, Stout and Bradley⁸ seems to us convincing that this tumor arises from the cells of the sheath of

6. Stout, A. P.: *Am. J. Cancer* **24**:751, 1935.

7. Foot, N. C.: *Am. J. Clin. Path.* **6**:1, 1936; *Arch. Path.* **30**:772, 1940.

8. Murray, M. R.; Stout, A. P., and Bradley, C. F.: *Am. J. Path.* **16**:41, 1940.

Schwann. Dr. N. C. Foot and Dr. J. F. Nonidez were of great assistance in the study of the nature of these tumors and in the final classification.

The close relation of the neurilemmoma of animal 7 to the sheath of nerves and of ganglions, on one hand, and to the circular muscle of the intestine, on the other, was of particular interest. A definite fusion between the circular muscle and the tumor was established at one point at least by the interlacing of their cellular constituents.

It is of interest that the tumors diagnosed as myoma were adjacent to the stomach and that those diagnosed as neurilemmoma were adjacent to the intestine. Stout⁸ and others⁹ have described many cases of neurilemmoma attached to the stomach in man. Neurilemmoma attached to the intestine is very rare in human material.

The liposarcoma of animal 5 was in close contact with the connective and adipose tissues of the intestine and not with its muscular coat. This was the only tumor that showed malignant characteristics. The fact that no positive transplants were obtained from these tumors may be attributed partly to the frequent use of old and nonhomogeneous animals as recipients.

With regard to metastasis, none could be definitely demonstrated. However, it is likely that in animal 7 the mesenteric nodules which showed the same structure of neurilemmoma as the tumor attached to the intestine were the result of a metastatic process. The mass found in the left lung of animal 5 was structurally different from the liposarcoma attached to the intestine and was probably of independent origin, although some relationship between the two growths cannot be entirely discounted. In animal 6 the tumor adjacent to the stomach was of different nature and definitely unrelated to the tumor attached to the intestine. The same thing can be said about the small adenoma of the thyroid of animal 7 and about the uterine tumor of animal 1. Multiple tumors are not rare in the guinea pig. The coexistence of a myoma of the stomach and an adenoma of the adrenal was described in a previous paper.¹

None of the tumors described in this paper was of glandular nature. All of them appear to have arisen from smooth muscle, nerve sheath or fat. The only reference to tumors in guinea pigs arising from nerves or nerve sheaths that we have found is that of Warren and Gates,¹⁰ who described a guinea pig with a "fibrosarcoma, probably of neurogenic origin, which had developed over the angle of the right jaw." This tumor appears to have been of a type different from those referred to as neurilemmoma in the present paper.

9. Gosset, A.; Bertrand, I., and Loewy, G.: *J. de chir.* **23**:577, 1924. Senturia, H. R.: *Am. J. Roentgenol.* **43**:61, 1940. Fuller, R. H.: *Arch. Path.* **32**:442, 1941.

10. Warren, S., and Gates, O.: *Cancer Research* **1**:65, 1941.

The age of the animals was undoubtedly a significant factor in the appearance of tumors. Of the 7 animals, 5 were over 6 and 2 over 4 years of age when the tumors were found.

In animal 5 the rate of growth of the tumor was noted for one hundred and thirty-four days between the time of operation and that of autopsy. The tumor grew especially fast at the point of incision.

The higher incidence of these tumors described in females (5 females and 2 males) is probably of no significance in view of the larger number of females in this colony.

In 4 of the 5 female animals, 1, 2, 5 and 7, the ovaries showed cystic degeneration and enlargement, but these changes are frequent in normal animals of this age.

SUMMARY

Five spontaneous tumors attached or adjacent to the stomach and three attached to the intestine of 7 guinea pigs are described. One animal had at the same time a tumor attached to the intestine and one adjacent to the stomach.

Four of the tumors of the stomach were classified as leiomyoma and one as lipoma. One of the tumors attached to the intestine was classified as liposarcoma and two as neurilemmoma. In 1 of the animals with neurilemmoma of the intestine, several nodules of a similar nature, possibly metastatic, were found in the mesentery, also an adenoma of the thyroid. In addition to the tumors named, 1 animal had a tumor of the cervix and another a fibrosarcoma of the thoracic wall.

All of the animals were senile, ranging in age from 4 to 6 years.

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EXPERIMENTAL HISTOPLASMOSIS IN MICE

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Histoplasmosis until very recently was considered a rare fungous infection. Indeed, the publication of only 7 cases in the thirty years following 1908 seemed to indicate its rarity. The first case of histoplasmosis to be reported was that described by Darling¹ in 1906. During the next two years 2 more cases were recognized by him.² He thought the causative agent was a protozoan parasite similar to *Leishmania* and therefore named it *Histoplasma capsulatum*. However, in 1934 De Monbreun³ succeeded in growing a hitherto undescribed fungus from the blood stream and organs of a child who died of the disease. He gave an accurate and complete description of the organism and with some misgivings named it *Histoplasma capsulatum*.

In its pathogenic form *H. capsulatum* occurs as a small, encapsulated yeastlike organism, which is engulfed by cells of the "reticuloendothelial" system and is rarely found free in the blood or the tissues. In the tissues and on mediums such as blood agar at 37 C. the organism has the structure of a yeast and divides by budding. On blood agar and other mediums at room temperature a mycelial form develops which is characterized by delicate cotton-white aerial hyphae which support large warty or tuberculate chlamydospores, some round and some piriform in aspect.⁴ De Monbreun³ was unsuccessful, as I also have been, in attempts to transform the mycelial form of the fungus to the yeastlike pathogenic form by cultural methods. Recently Conant has succeeded in this.^{4a}

The most common clinical manifestations of histoplasmosis are low grade irregular pyrexia, leukopenia, anemia, splenomegaly, hepatomegaly, lymphadenopathy, and ulcerated granulomatous lesions in the skin, the oral and pharyngeal mucous membranes and the gastrointestinal tract. Histologic examination reveals granulomatous lesions of the lungs, liver, spleen, adrenals, lymph nodes, bone marrow and other tissues. The granulation tissue consists chiefly of large mononuclear phagocytes, which contain the small yeastlike form of the fungus in their cytoplasm.

From the Department of Pathology of the University of Michigan.

1. Darling, S. T.: J. A. M. A. **46**:1283, 1906.
2. Darling, S. T.: Arch. Int. Med. **2**:107, 1908.
3. De Monbreun, W. A.: Am. J. Trop. Med. **14**:93, 1934.
4. (a) Conant, N. F.: J. Bact. **41**:563, 1941. (b) De Monbreun.³

A delicate meshwork of young connective tissue and a few lymphocytes are usually a part of the granulation tissue.

Between November 1938 and January 1941, 4 cases of histoplasmosis were seen at the University Hospital. In 2 of these the diagnosis was made on histologic examination of tissues obtained at autopsy. In another the diagnosis was first made on examination of biopsy specimens from a tonsil and an external auditory canal. The diagnosis was confirmed by culture of *H. capsulatum* from lymph nodes removed during life and by culture of the same organism from the adrenals after death. In the fourth case the diagnosis was made on biopsy of an indurated ulcer of the naris which had perforated the nasal septum. On a second occasion biopsy of this lesion with culture on dextrose-tartaric acid medium resulted in the abundant growth of the mycelial form of *H. capsulatum*. Work with the last-mentioned culture constitutes the basis for this report, in which the results of studies on histoplasmosis induced in mice are described.

TRANSFORMATION OF THE MYCELIAL TO THE YEASTLIKE FORM IN MICE AND PRODUCTION OF HISTOPLASMOSIS

In several experiments the mycelial form of *H. capsulatum* was grown on dextrose-tartaric acid medium^{4a} or on Sabouraud's medium for at least sixty-nine days at room temperatures. The fungus was then ground thoroughly in dextrose infusion broth in a small sterile mortar. (The suspension was examined microscopically for evidence of the yeastlike form of the fungus, and none was found.) With the supernatant fluid from such a suspension, white mice, 3 to 5 weeks old, were given intravenously or intraperitoneally 0.05 and 0.2 cc. doses. For intravenous injections, the tail vein was used.

In the first experiment 0.2 cc. of a suspension of a ninety-four day old culture of the organism from dextrose-tartaric acid medium was injected intravenously into each of 5 half-grown mice. All died in from twenty-two to one hundred and twelve days. In several instances the abdomen became distended a few days before death. At autopsy, this was found to be due to a striking enlargement of the spleen. In one instance the spleen measured 35.0 by 7.5 by 3.5 mm. and weighed 0.42 Gm. These figures were frequently exceeded in later experiments, one spleen weighing 1.43 Gm.⁵ The spleen varied in color from a uniform gray-red

4a. The formula for dextrose-tartaric acid medium is as follows:

Dextrose	250 Gm.
Tartaric acid.....	25 Gm.
Distilled water.....	500 cc.

Autoclave. Cool slowly in a water bath to avoid any precipitate. (From Henrici, A. T.: *Molds, Yeasts and Actinomycetes*, New York, John Wiley & Sons, Inc., 1930, p. 25.)

5. The weights of the spleens and the livers and the weights of 6 normal adult mice of the stock used in these experiments were as follows:

	Range of Weight	Av. Weight
Mice	36.1 -42.8 Gm.	39.3 Gm.
Spleens	0.10- 0.20 Gm.	0.14 Gm.
Livers	2.00- 2.70 Gm.	2.35 Gm.

to a pale gray, and well circumscribed dull yellow areas of necrosis could frequently be seen through the capsule. The cut surface varied from a glistening, slightly mottled grayish red to gray and in several instances showed irregular dull yellow necrotic areas. The liver was frequently enlarged but to a less extent than the spleen. In this group of animals the greatest hepatic weight was 3.3 Gm., while the largest liver seen in later experiments weighed 4.4 Gm. The livers commonly appeared to be somewhat paler than usual both externally and on the cut surfaces. The largest liver in this group had an irregularly mottled brownish red appearance but showed no areas of focal necrosis visible to the eye. Grossly, the lungs were somewhat congested, and a slight to moderate increase in density was usually present. In severe cases the lungs were a mottled dark red and gray-red on the cut surfaces.

Microscopically, one could see in many tissues large mononuclear phagocytes with intracytoplasmic small round or oval yeastlike bodies. Each body contained a central round or oval chromatin mass which stained fairly well with most nuclear dyes. With high magnifications, a small clear round vacuole was seen within the chromatin. It was frequently centrally placed in the round forms but was usually eccentrically placed in the oval forms. The chromatin mass was surrounded by a clear capsule which failed to stain with any dye which we used.⁶

In the lungs the interalveolar septums were greatly widened by parasitized cells, in some places to such an extent that little or no alveolar space remained. The source of these cells could not be determined with certainty. They could have been immobilized and heavily parasitized monocytes, heavily parasitized histiocytes, parasitized endothelial leukocytes or, as they actually appeared to be in some instances, strikingly hyperplastic and parasitized cells of the vascular endothelium itself. Rarely a parasitized cell was found free in an alveolus. Heavy parasitization of the Kupffer cells was seen in the liver (fig. 1A). Many of the sinusoids were distended by parasitized cells, and in the more severe involvements the cords of liver cells showed varying degrees of compression with complete necrosis of the cells at some points. In the tissue surrounding the areas of necrosis a few polymorphonuclear leukocytes were present. The walls of many of the sublobular veins were thickened and heavily infiltrated with parasitized phagocytes, and the endothelium in many places showed the same type of hyperplasia and parasitization that had been noted in the lungs. Moderate numbers of parasitized cells were intermixed with lymphocytes and a few plasma cells in the connective tissue of the portal canals. The manifestations of histoplasmosis in the spleen varied from the presence of moderate numbers of parasitized cells attached to the walls of the sinusoids (fig. 1B) to the filling of the red pulp with parasitized cells, marked hyperplasia of reticuloendothelial cells and complete effacement of the usual splenic structure, with almost complete disappearance of lymphocytes and megakaryocytes. In the most severely affected areas, caseation necrosis was present, and a few polymorphonuclear cells were present in the

6. For routine use we have found a fairly heavy alum-hematoxylin stain and a light eosin stain entirely satisfactory. Of the many other stains that we have used, the Goldner modification of the Masson trichrome stain (*Am. J. Path.* 14:237, 1938) has been the best, probably as a result of the high degree of translucency of sections so stained. The organisms in tissues are slightly acid fast, and some of the organisms retain the crystal violet in Gram's stain if the decolorization has not been carried too far.

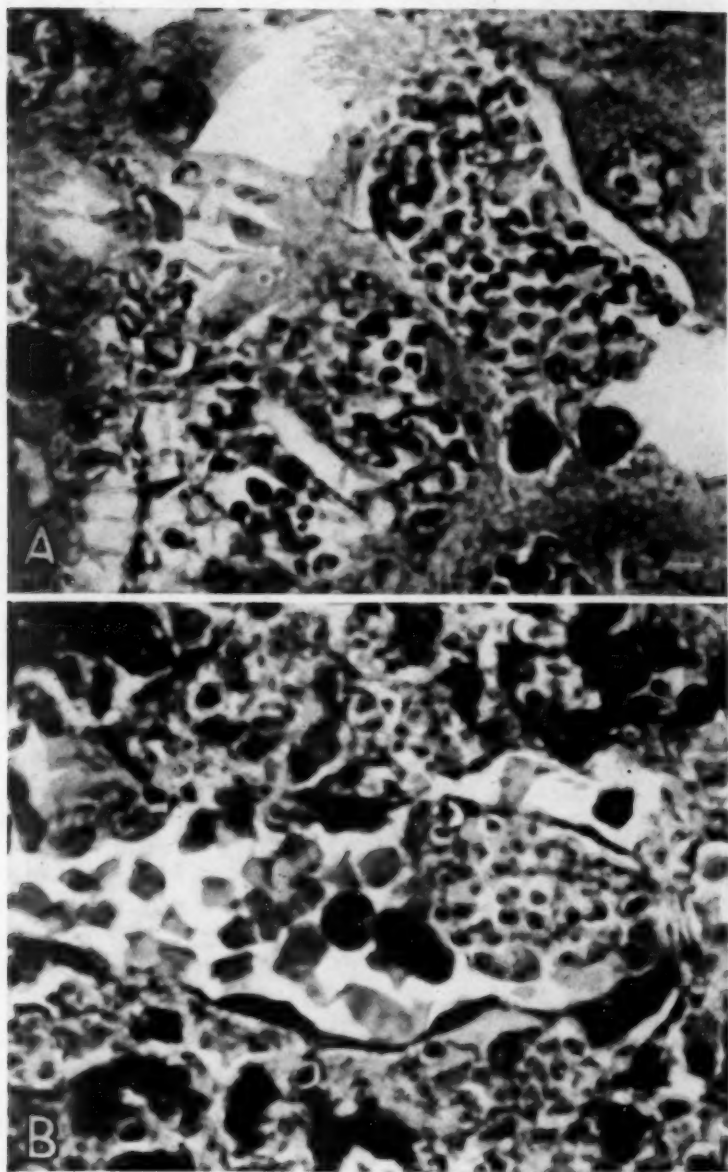


Fig. 1.—*A*, section of mouse liver in which the Kupffer cells are heavily parasitized by the yeastlike form of *H. capsulatum*. The sinusoids are almost filled by the parasitized cells. Hematoxylin and eosin; $\times 1,860$. *B*, heavily parasitized endothelial leukocyte attached to the wall of a splenic sinusoid. Note the clear halo or capsule outside the chromatin mass of each organism. Numerous parasitized cells are also visible outside the wall of the sinusoid. Hematoxylin and eosin; $\times 1,860$.

surrounding tissue. The areas of caseation necrosis were frequently surrounded with heavily parasitized macrophages. In some instances these areas were somewhat wedge shaped, suggesting that occlusion of a small artery might be the immediate cause. The finding of small vessels completely or partially occluded by parasitized endothelial leukocytes or monocytes lends some support to this possibility. Lymph nodes were frequently involved, the lesions varying from the presence of one or more parasitized monocytes in a peripheral sinus to the almost complete replacement of the node with parasitized cells and occurrence of focal areas of necrosis. The yeastlike organisms were frequently found in the cytoplasm of monocytes in the peripheral blood (fig. 2A).

The organs most commonly and most severely involved are those which have been described, but nearly all other organs and tissues may at times be affected. Parasitized macrophage cells in large numbers have been found in the bone marrow, the subcutaneous tissues, the perirenal and retroperitoneal connective tissue, the pancreas, the submucosa and the subserosa of the stomach and in the kidney. In the kidney, parasitized cells were most commonly seen in the lumens of the capillaries of the glomerular tufts. They were found also in varying num-

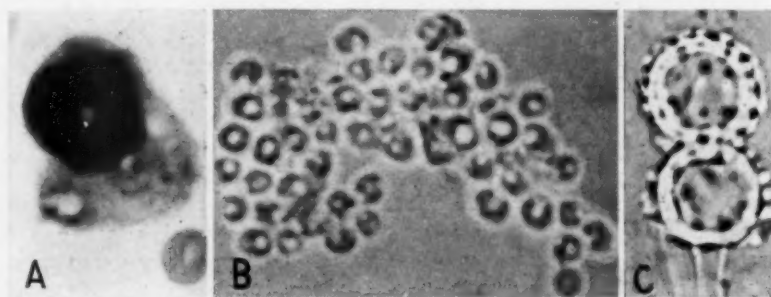


Fig. 2.—A, four yeastlike forms of *H. capsulatum* are shown in a monocyte from the circulating blood. The organisms are all somewhat out of focus as the result of an attempt to show all of them in one photograph. Wright's stain; $\times 1,860$. B, photograph of a smear of a six day culture of the yeastlike form of *H. capsulatum* which had been grown on a rabbit blood agar slant at 37 C. Aniline blue stain; $\times 1,860$. C, photograph of two of the characteristic tuberculate chlamydospores found in a suspension of the mycelial form of *H. capsulatum*. The fungus had been cultured on dextrose-tartaric acid medium. Unstained; $\times 1,860$.

bers in the intertubular capillaries. On rare occasions they were sufficiently numerous to cause compression and necrosis of the tubules.

From these results it seemed that the intravenous injection of the mycelial form of *H. capsulatum* was at once an excellent method of converting the fungus to the yeastlike or pathogenic form and of producing histoplasmosis in mice. However, my attempts to repeat this experiment gave much less satisfactory results. One of two experiments will illustrate the results. A suspension of the mycelial form of *H. capsulatum* of the same type that had been used previously was injected into 13 mice. In this experiment the fungus had been kept in broth for one hundred and twenty-eight days at room temperatures and had been on dextrose-tartaric acid medium for the previous thirty-four days. Only 3 mice

gave evidence of infection with the yeastlike form and in only 2 of the 3 was the infection extensive. Most of the animals in this group lived until they were killed, one hundred and six days after inoculation.

A final experiment of this type was done, in which a culture of the mycelial form of *H. capsulatum* that had been growing on Sabouraud's medium for sixty-nine days was used. The organism had been planted on Sabouraud's medium in the yeastlike form. None of the yeastlike form of the organism could be seen in the dextrose infusion broth suspension that was employed. The results closely paralleled those of the first experiment. Eight mice were given 0.05 cc. of the suspension, and all died in from seventeen to thirty-six days, heavily infected with the yeastlike form of the organism.

To complete the full cycle with *H. capsulatum* and to fulfil completely Koch's somewhat time-worn postulates, the organism was easily and regularly recovered from the mice. The dead mouse was placed in a 4 per cent solution of formaldehyde for ten to fifteen minutes. Sterile instruments were used to open the abdomen, and the spleen was removed to a sterile Petri dish. A freshly cut surface of the spleen was streaked on a suitable culture medium. Streaking of an infected spleen on rabbit blood agar and incubating at 37 C. resulted almost invariably (contamination was rare) in the growth of a pure culture of the yeastlike form of *H. capsulatum*. The colonies were usually first visible as slightly elevated "pinpoints" on the third to the fifth day. From the fifth to the tenth day they enlarged to form moist gray-white dome-shaped colonies which were easily removed from the medium. After the tenth day the growth became dry and rough in appearance, with short spinelike aerial hyphae projecting perpendicularly from the surface. After the tenth day the colonies assumed a dirty grayish red color. Smears of the organisms made on the third to the fifth day showed a small budding yeast form slightly larger than those found in the tissues and without a distinct capsule (fig. 2 B). The organisms were otherwise morphologically similar to those in the tissues. Smears made after longer periods of incubation revealed organisms of increasing size and the formation of short mycelial forms, which became more numerous from day to day. Still later, branching, septate mycelial forms predominated. The organism could easily be maintained in the yeastlike form when it was subcultured on the same medium at four to fourteen day intervals.

On Sabouraud's or on the dextrose-tartaric acid medium, almost colorless, dry and slightly rough-appearing colonies could usually be seen by the fifth to the eighth day along the streak line produced by the cut surface of an infected spleen or on subculture of the yeastlike form grown on rabbit blood agar. By the tenth to the fifteenth day numerous delicate cotton-white aerial hyphae were visible to the naked eye. During the next few weeks the colonies increased in size and at first grossly resembled tiny bits of white cotton. Later a light brownish color could be seen in the older central part of the colony, and this color gradually migrated toward the periphery. Microscopic examination of these colonies after approximately thirty days of growth showed a dense interlacing network of very delicate septate mycelial strands supporting fruiting bodies of several types. Of these, the important ones from the standpoint of positive identification of the fungus were large round or piriform bodies attached terminally or by short lateral stalks to the aerial hyphae. These had a dense outer membrane, on the surface of which numerous rounded knobs were present, resulting in a warty or tuberculated appearance (fig. 2 C). The work of Conant^{4a} would

seem to show that these bodies are chlamydospores, but Moore⁷ and De Monbreun³ expressed the belief that they are asci or at least ascus-like bodies. All authors have agreed that they are characteristic of a species of fungus, *H. capsulatum*, and some authors have established a new genus, *Histoplasma*, for this fungus.

RESULTS OF THE INTRAPERITONEAL INJECTION OF THE MYCELIAL FORM

Having demonstrated the efficacy of the intravenous method for the transformation of the mycelial to the yeastlike form of *H. capsulatum* and for the production of the disease in mice, the intraperitoneal route of injection was next tested. De Monbreun³ described the transformation by this procedure in mice. A dextrose infusion broth suspension of the mycelial form of *H. capsulatum* was injected in 0.2 cc. amounts into the peritoneal cavities of 5 white mice 3 weeks old. None of the animals died spontaneously. Two of them lived until they were killed three hundred and eighty-six days after inoculation, at which time there was neither gross nor microscopic evidence that the fungus had been introduced into the peritoneum. The other 3 mice had been killed nine, twenty-three and thirty days after inoculation. In each of these animals a very few macrophages, parasitized with the yeastlike form of the fungus, were found in the omentum, in the mesentery and in the peritoneum covering the posterior part of the abdominal cavity. In 1 mouse the omentum was densely infiltrated with large mononuclear phagocytes, but only three parasitized cells could be found in sections. At the time of autopsy of the mouse that was put to death on the thirtieth day, a sterile loop was passed over the peritoneal surfaces and then streaked on a blood agar slant. A pure culture of the yeastlike form of the fungus grew along the streak lines. We can thus confirm De Monbreun's³ findings relating to the transformation of the fungus in the peritoneum of the mouse, but infection of the peritoneum does not appear to result in disseminated histoplasmosis.^{7a}

INDUCTION OF HISTOPLASMOSIS WITH THE YEASTLIKE FORM OF *H. CAPSULATUM*

Several experiments were done to find the most efficient method for inducing histoplasmosis in mice as well as to control the dosage of the organism so that the mice would live for a reasonable length of time after infection. Intravenous injection of a dextrose infusion broth suspension of ground-up fragments of spleen and liver, or of spleen alone, from mice that had been previously infected, proved to be an efficient and reliable method of producing the disease. To further standardize the procedures, mice only 3 weeks old were given 0.2 cc. quantities of the suspension. In one such experiment the 4 animals which had been given injections were all found dead on the seventeenth day. Such uniformity of results was, however, unusual. The majority of the mice treated

7. Moore, M.: (a) *Ann. Missouri Bot. Garden* **22**:335, 1935; (b) *Am. J. Trop. Med.* **21**:627, 1941.

7a. M. Tager and A. A. Liebon (*Yale J. Biol. & Med.* **14**:469, 1942) have recently reported the production of generalized histoplasmosis in mice by intraperitoneal injection of the mycelial form of *H. capsulatum*.

in this way died in from eight to twenty-eight days with evidence of overwhelming infection with the yeastlike form of *H. capsulatum*. Rarely a mouse would live for a much longer period. A mouse that was killed on the three hundred and seventy-first day after infection showed heavily parasitized macrophages in nearly every tissue of the body, and the spleen weighed 1.3 Gm., while the liver weighed 4.0 Gm. Another animal surviving for three hundred and seventy days showed no enlargement of the spleen or of the liver and histologically showed no evidence of histoplasmosis.

On two occasions, subcutaneous lymph nodes from one of our patients with histoplasmosis were ground with dextrose broth or Locke's solution. The resulting suspensions were injected intravenously in 0.05 or 0.1 cc. amounts into white mice 4 to 5 weeks old. One of the mice, put to death thirty-six days after inoculation, showed widespread parasitization of cells of the macrophage group. The remaining mice died from three to nine months after inoculation, all showing extensive lesions such as were described in earlier paragraphs. The spleens of 2 of these mice were enormous, weighing 1.20 and 1.43 Gm., respectively.

It was found possible to induce histoplasmosis by the injection of the yeastlike form of *H. capsulatum* grown on rabbit blood agar slants at 37 C. Since the use of cultured organisms seemed to offer most in the way of ease and accuracy of control of dosage, several experiments were performed to find whether it was possible to control the duration of the disease by varying the number of organisms injected.

In a typical experiment a four day growth of the yeastlike form of *H. capsulatum* was washed from the surface of a rabbit blood agar slant with 3 cc. of dextrose infusion broth. The organisms washed off easily and well. The number of organisms in the suspension was determined in a suitable counting chamber, 135,000 organisms per cubic millimeter being found. Preliminary experiments of this type had given a rough indication of the desirable number of organisms in the infecting dose. Hence the suspension was diluted 1 to 5 and 1 to 10 with the broth. The original suspension and each of the dilutions of the suspension were injected intravenously into mice, which had been divided into groups of 4. Since 0.2 cc. was injected into each mouse, doses of approximately 27,000,000, 5,400,000 and 2,700,000 organisms per mouse resulted.

All of the mice given the largest dose were found dead on the sixth day. Those receiving 5,400,000 organisms survived six, ten, eleven and eleven days, an average of nine and five-tenths days, while the mice receiving 2,700,000 organisms survived eleven, fourteen, fourteen and fifteen days, an average of thirteen and seven-tenths days. These results show that by varying the dose of any given suspension the period of survival of the mice can be controlled to some extent. But in other experiments, in which other suspensions were used, doses of approximately 100,000 organisms have killed all of the mice in an average of eleven days, and doses of approximately 1,000,000 organisms have caused death in but 2 of 3 mice after twelve and eighteen days. In this series, the third mouse, killed one hundred and four days after inoculation, showed no evidence of the disease. On two occasions the yeastlike form of *H. capsulatum* has been found in macrophages of the spleen, the liver or the lungs more than three hundred days after the intravenous injection of approximately 2,600 organisms. In 1 of these animals the extent of the parasitization of the macrophage system was considerable, while in the other mouse only a few organisms were found and death probably resulted from another cause.

COMMENT

These experiments have shown that the white mouse is susceptible to *H. capsulatum*, which when injected intravenously produces in them a highly characteristic and usually fatal infection resembling in many ways the disease caused by this organism in man. In each case the yeast-like pathogenic form of the fungus invades cells of the large mononuclear phagocytic type, which are frequently referred to as cells of the reticulo-endothelial system. The fungus causes striking hyperplasia of these cells and, as a result of the abundance of these cells in the spleen and the liver, produces splenomegaly and hepatomegaly of varying degree. By controlling the dose of the fungus, one can induce in mice a fulminating and rapidly fatal infection or a more protracted one. As to man, Dodd and Tompkins⁸ and Agress and Gray⁹ have seen the rapidly fatal course of the infection in young children; in adults, as in the case of Riley and Watson¹⁰ and that of Hansmann and Schenken¹¹ and the 4 cases of Parsons, Zarafonitis and Hamff,¹² there is some evidence that the infection may be present from a few months to several years.

While De Monbreun³ and I have found that the yeastlike pathogenic form of the fungus can be obtained from the peritoneum of the mouse following the intraperitoneal injection of the mycelial form, generalization of the infection has not occurred when this type of inoculation has been employed by me. It has been found possible to produce this transformation by intravenous injection of the mycelial form, with resulting widespread dissemination of the pathogenic form in the mouse. As has been pointed out, a fatal infection frequently but not always results from this procedure.

Suspensions of spleen and liver from previously infected mice regularly produce generalized histoplasmosis in young mice following intravenous inoculation. On two occasions suspensions of lymph nodes removed surgically from 1 of our patients with histoplasmosis produced generalized and fatal histoplasmosis in all the mice inoculated intravenously. The use of this method would seem to have considerable value in the recovery of *H. capsulatum* from certain human lesions, particularly those in which there is considerable secondary infection. In culture, *H. capsulatum* grows rather slowly, and contaminating bacteria or molds frequently render isolation of the organism difficult.

8. Dodd, K., and Tompkins, E. H.: *Am. J. Trop. Med.* **14**:127, 1934.

9. Agress, H., and Gray, S. H.: *Am. J. Dis. Child.* **57**:573, 1939.

10. Riley, W. A., and Watson, C. J.: *Am. J. Trop. Med.* **6**:271, 1926.

11. Hansmann, G. H., and Schenken, J. R.: *Am. J. Path.* **10**:731, 1934.

12. Parsons, R. J.; Zarafonitis, C., and Hamff, L. H.: *Histoplasmosis of Darling, with the Report of Four Additional Cases*, to be published.

The mouse will sometimes inhibit or destroy the contaminants, and a pure culture of *H. capsulatum* may then be obtained from the spleen or the liver of the mouse.

Other lower animals have been found susceptible to *H. capsulatum* in a varying degree. Hansmann and Schenken¹¹ found the dog and the rat to be fairly susceptible, the rabbit and the guinea pig to be less so. De Monbreun³ found monkeys to be very susceptible and puppies and mice to be less so. In the present work a high degree of susceptibility to intravenous injection of the organism was demonstrated in mice. Intraperitoneal inoculation, on the other hand, has resulted in an evanescent localized infection only. Recently Moore^{7b} successfully inoculated the chorioallantoic membrane of the chick with the mycelial form. De Monbreun¹³ found *H. capsulatum* occurring naturally in a dog. In 1922 Sangiorgi¹⁴ described spontaneous blastomycosis in a rat, *Mus rattus*. His description of the disease and drawings and photomicrographs of the organisms render it probable that he was dealing with an organism closely related to *H. capsulatum*. The next year Shortt,¹⁵ in India, found many of his experimental mice infected with what he called *Cryptococcus muris*. Again, his description of the organisms strongly suggests *H. capsulatum*. Neither Sangiorgi nor Shortt was able to culture the organism. Finally, Levine, Dunlap and Graham¹⁶ described and photographed what appears to be the same type of organism in the spleen, the liver and the lung of a ferret. The organism was not cultured, and only by cultural methods can it be positively identified. This is well brought out by the disease known as farcy in the Mediterranean basin. The parasite in this disease (*Cryptococcus farciminosus*) closely resembles the yeastlike form of *H. capsulatum* when seen in the tissues. It invades the macrophage cells as does the latter. Cultural studies, however, reveal wide fundamental differences between the two fungi.³

SUMMARY

In mice intravenous and intraperitoneal injections of the mycelial form of *H. capsulatum* result in the transformation of the organism to the yeastlike or pathogenic form.

The intravenous injection of the mycelial form usually, and that of the yeastlike form regularly, results in generalized and fatal histoplasmosis in young white mice.

13. De Monbreun, W. A.: *Am. J. Trop. Med.* **19**:565, 1939.

14. Sangiorgi, G.: *Pathologica* **14**:493, 1922.

15. Shortt, H. E.: *Indian J. M. Research* **10**:908, 1922.

16. Levine, N. D.; Dunlap, G. L., and Graham, R.: *Cornell Vet.* **28**:249, 1938.

The gross and the microscopic changes in histoplasmosis of mice, as well as the important cultural characteristics of *H. capsulatum*, are described.

It is believed that certain of the procedures described may be of use in the isolation of the fungus in human cases of the disease.

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FIBROSARCOMA OF ARACHNOIDAL ORIGIN WITH METASTASES

REPORT OF FOUR CASES WITH NECROPSY

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The question of metastases from any primary intracranial tumor has been a subject of great interest both to neurosurgeons and to pathologists. The type of intracranial tumor to be considered in this communication is the tumor commonly observed arising from the arachnoid composed of elongated spindle-shaped cells, frequently forming whorls or palisades and having scattered focal areas of calcification. Various names have been used for this tumor—"dural endothelioma," "arachnoidal fibroblastoma," "meningioma" and "meningeal fibroblastoma." In this paper the term "arachnoidal fibroblastoma" will be used because we believe the cases here reported offer additional evidence for the mesodermal origin of this tumor for which this term is predicated. After a careful review of the literature we have found but 4 tumors related to the arachnoidal fibroblastoma group, or at least tumors apparently having the same cell of origin as that group, which have metastasized (Klebs¹; Lindner²; Cushing and Eisenhardt^{3a}; Jurow⁴). Another case is reported,⁵ but the histologic description of the tumor leaves considerable doubt concerning the accuracy of the diagnosis. The 4 well established cases were as follows:

Klebs¹ was the first to report a tumor of arachnoidal origin with visceral metastases, which he called a "miliary form of endothelioma" of the pia mater. Numerous large and small gray nodules of tumor tissue were noted in the lungs at necropsy, which were regarded as

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1. Klebs, E.: *Die allgemeine Pathologie*, Jena, Gustav Fischer, 1889, vol. 2, p. 628.

2. Lindner, E.: *Ztschr. f. Heilk.* **23**:118, 1902; cited by Jurow.⁴

3. Cushing, H., and Eisenhardt, L.: *Meningiomas*, Springfield, Ill., Charles C. Thomas, Publisher, 1938, (a) p. 716; (b) p. 49; (c) p. 50; (d) p. 69.

4. Jurow, H. N.: *Arch. Path.* **32**:222, 1941.

5. Dérévici, M.; Ionescu, E. I., and Smilovici, L.: *Bull. Soc. roum. de neurol., psychiat., psychol. et endocrinol.* **18**:14, 1937.

metastases from the primary locus in the brain. Histologic examination of the tumor showed the same type of growth in the lungs as was observed in the brain.

The second instance of metastasis from a tumor of arachnoidal origin to a distant organ was reported by Lindner.² The patient was a 63 year old man who died from an intracranial tumor, and at necropsy a second tumor was found involving the urinary bladder. Histologic study of the tumors from the meninges and the urinary bladder revealed the same histologic type of growth. The tumors were rapidly growing ones, and the diagnosis of "endothelioma" was given.

A third case was reported by Cushing and Eisenhardt^{3a} in their classic monograph dealing with 313 cases of arachnoidal fibroblastoma. The case reported by these authors was that of a tumor which recurred after seventeen operations over a period of thirteen years. At necropsy three small tumor nodules were observed in the upper lobe of the right lung, the largest measuring 2 to 3 cm. in diameter. The tumor removed at the first operation was composed of spindle-shaped cells with numerous mitotic figures and areas of hyaline degeneration. At all subsequent operations the tumor tissue removed showed cuboidal or columnar cells with a striking epithelial-like arrangement of the cells and frequent mitotic figures. Since no other tumor was noted at necropsy, the nodules of tumor in the lung were regarded by these authors as metastases from the primary locus in the meninges. The case was regarded as that of a malignant type of arachnoidal fibroblastoma which had metastasized to the lung.

The 3 tumors cited and reported as primary meningeal tumors with metastases all departed remarkably from the usual well differentiated type of arachnoidal fibroblastoma. From a purely histologic standpoint, these tumors completely satisfied the criteria for a malignant tumor, namely, invasion, anaplasia, rapid growth as indicated by the presence of mitotic figures and metastases.

The case recently reported by Jurow⁴ of arachnoidal fibroblastoma with metastases is of special interest since the histologic criteria for the diagnosis of a malignant tumor, so conspicuous in the 3 cases aforementioned, were absent. In this case, the intracranial tumor and the metastatic tumor in the lung were incidental findings at necropsy, for the patient died of lobar pneumonia with no signs or symptoms to suggest intracranial disease. The histologic examination of the tumor removed from the meninges revealed a not unusual type of well differentiated arachnoidal fibroblastoma with psammoma bodies. Anaplasia, necrosis and mitotic figures were absent, so that the metastasis in this case was the sole criterion for regarding the tumor as malignant. The tumor noted in the lung was well circumscribed and histologically identical

with the tumor in the brain. This case clearly indicates that apparently benign arachnoidal fibroblastoma, at least as far as it can be judged from objective histomorphologic criteria, can give rise in rare instances to metastases in distant organs. Analogous examples of tumors benign as far as histologic criteria are concerned which in rare instances have produced metastases are leiomyoma of the uterus⁶ and adenoma of the thyroid.⁷ We feel that the diagnosis of such tumors as benign is definitely excluded when metastases occur even though histologic examination of the primary growth discloses a picture indistinguishable from that of similar tumors showing no metastases. The diagnosis of the aforementioned tumors therefore should be leiomyosarcoma of the uterus and carcinoma of the thyroid. By the same line of reasoning the diagnosis of the tumor in the case of Jurow should be sarcoma and even more correctly fibrosarcoma.

Malignant transformation of arachnoidal fibroblastoma has been reported without metastases.⁸ The diagnosis in each instance depended solely on the histologic evidence of malignancy. These reports of malignant transformation of arachnoidal fibroblastoma illustrate well that this tumor may become locally malignant. The diffuse intracranial neoplasms reported as sarcoma by Bailey⁹ in 1929 and Bailey and Bucy¹⁰ in 1931 and recently by Hsu¹¹ are a distinctly different group from the arachnoidal fibroblastoma group, a point which Cushing and Eisenhardt^{3c} emphasized in their monograph. For this reason, a further consideration of this type of intracranial sarcoma is not indicated in this communication.

It is the purpose of this paper to report 4 additional intracranial tumors primary in the leptomeninges and histogenetically related to arachnoidal fibroblastoma which metastasized. These tumors exhibited histologically many of the general characteristics of fibrosarcoma. They are of special significance, therefore, because they add additional evidence that the arachnoidal cells of the leptomeninges are of mesodermal origin.

REPORT OF CASES

CASE 1.—Malignant fibroblastic tumor resembling arachnoidal fibroblastoma with metastases to the lungs, the pleura, the abdominal lymph nodes and a lumbar vertebra.

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9. Bailey, P.: Arch. Surg. **18**:1359, 1929.

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History.—E. R., a housewife aged 33, was admitted to the Barnes Hospital Oct. 19, 1935. The family history was not relative. In her own history the two significant points were that as a child she received a severe kick on the top of the head and was unconscious for a short period. There resulted from this accident a deep scar over the region of the longitudinal sinus.

The patient's symptoms began in April 1935, when she first had pain in the right side of her head and buzzing in her right ear. Three weeks before admission, she was noticeably forgetful, and attacks of vomiting were associated with her headaches.

On neurologic examination the positive findings were: tenderness in the right parietal region; choked disks with hemorrhages in both fundi; paralysis of the left side of the face, of the central type; a lessened abdominal reflex on the left as compared with that on the right; bilateral Gordon and Oppenheim signs. Perimetric studies showed the visual fields normal. Roentgen plates of the skull revealed slight pressure markings. Although the neurologic symptoms suggested a lesion on the right side, they were not definite enough to warrant operation without first making a ventriculogram. A perforator opening made on the right exposed a grayish mass of tumor tissue. Air put into the ventricle on the opposite side demonstrated a tumor in the right parieto-occipital region. Craniotomy was performed on the right side immediately after the taking of the ventriculogram and revealed a sharply demarcated, encapsulated soft tumor, which was attached to the falx and the longitudinal sinus. It was believed that the tumor was removed completely. However, because there was some question whether there might be some tumor cells left along the longitudinal sinus, a decompression was left under the temporal muscle. The tumor tissue removed weighed 41 Gm. High voltage roentgen therapy was given before the patient was discharged, November 13.

The tumor removed at operation was moderately cellular. The cells were oval or spindle shaped and contained small, elongated, moderately chromatic nuclei. Numerous mitotic figures were noted throughout the section. In certain areas the cells were arranged end to end to form sheets of cells, which were arranged in parallel formations, and in some instances the sheets of cells showed a slight tendency to converge toward a common center and to form concentric layers (fig. 1A). In other parts of the tumor the cells showed no characteristic arrangement. Scattered throughout the section were many areas of loose hyalinized connective tissue, devoid of tumor cells. Focal areas of calcification with irregularly outlined lobulated edges were scattered in the connective tissue and in some places between the tumor cells. A section stained by Mallory's phosphotungstic acid-hematoxylin method revealed a small amount of fine, brownish red-staining collagen between the tumor cells and a few fine, blue-staining intracellular fibrils. A section prepared with the Perdrau method for reticulum revealed abundant fine reticulum between the tumor cells. In those areas of the tumor where the cells were arranged in sheets and tended to form concentric layers, reticulum fibers were noted between the sheets of cells, giving to the section a reticulum pattern characteristic of arachnoidal fibroblastoma (fig. 1B).

The pathologic diagnosis was "malignant arachnoidal fibroblastoma," and a guarded prognosis was given.

The patient was carefully followed. She was operated on at an outside hospital in May 1936 for pelvic inflammatory disease, and a complete hysterectomy was performed. In October 1936 she had a severe convulsion. At this time she again had pathologic reflexes on the right side, and there was slight bulging of the decompression wound. The patient was readmitted to Barnes Hospital October

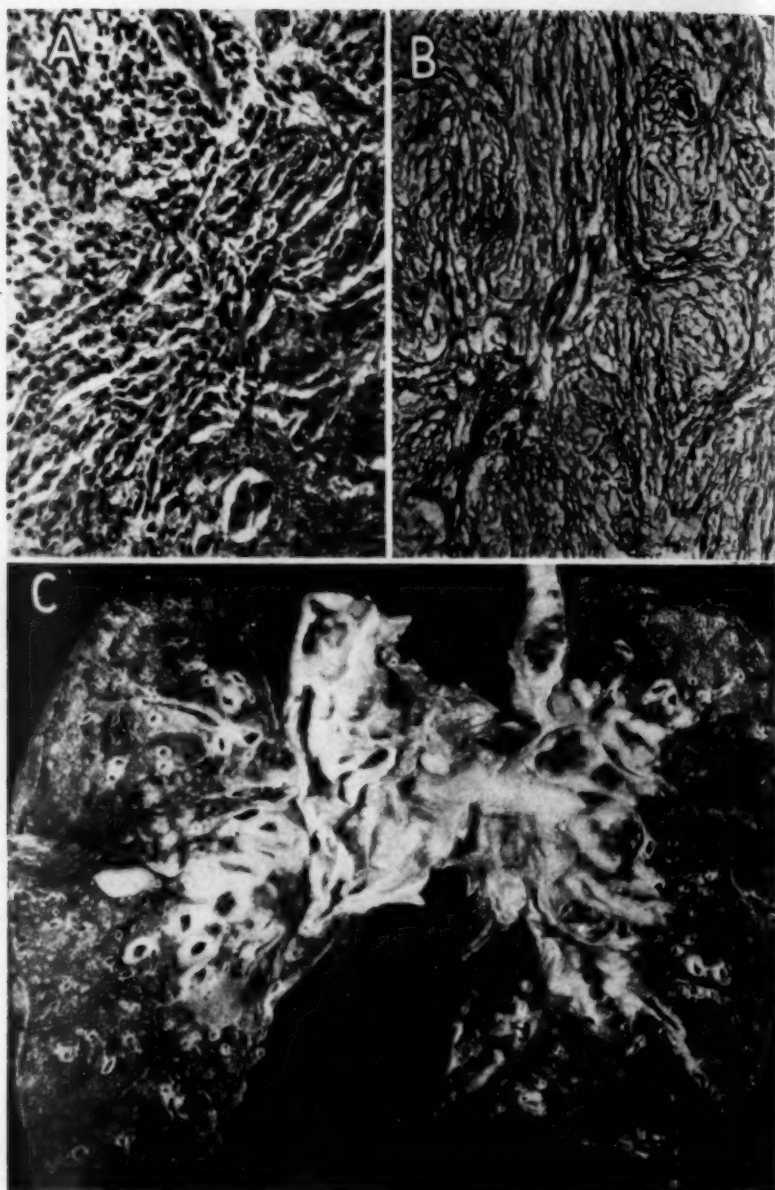


Fig. 1.—(case 1), *A*, section of the tumor removed at the first operation, showing arrangement of the cells into sheets with a slight tendency to form concentric layers strongly suggestive of arachnoidal fibroblastoma. Hematoxylin and eosin stain; $\times 140$.

B, large amounts of intercellular reticulum clearly outlining the sheets of tumor cells. Note the concentric arrangements of the reticulum bundles in some areas, which is characteristic of arachnoidal fibroblastoma. Perdrau impregnation method for reticulum; $\times 140$.

C, horizontal section through the lungs. Note the large masses of tumor tissue involving the hilus of each lung and the lymph nodes of the mediastinum. The unusually prominent bronchi are heavily infiltrated with tumor.

8. Neurologic examination at this time revealed homonymous hemianopia, pathologic reflexes and changes in the eyegrounds. There was a question whether the symptoms were caused by the old scar or whether there was recurrence of the tumor. An exploration was made on October 10, through the old operative wound. There was a recurrent tumor surrounded by a dense fibrous scar. The tissue removed weighed 6.5 Gm. There was a slight postoperative complication in the form of necrosis of the skin near the median line at the site of the old scar, which had to heal by granulation. The patient was discharged October 28.

Histologic examination of the tumor removed at this operation revealed essentially the same type of tumor as was noted at the first operation. Generally, however, there was lessened tendency for the cells to be arranged in sheets and parallel formations, and focal calcification was not present. Osteomyelitis developed in a small area at the site of the granulation tissue, and the involved bone was removed Feb. 24, 1937. Two months later the patient was readmitted because osteomyelitis had again become active. Operation for the osteomyelitis was successful, but at that time there was some question whether there was not recurrence of the tumor.

The patient was readmitted in September. At that time there was marked herniation of the scar at the site of the old osteomyelitis and there was clearly recurrence of the tumor. Because there still was an open granulating area, Dr. Barrett Brown first applied a skin graft on this area. When the area was completely healed (March 1938), craniotomy was done a third time and revealed an enormous recurrent tumor mass beneath the old scar. The tumor removed weighed 91 Gm., but there was more tumor tissue in the ventricles. In spite of every available care, the patient died March 17, 1938, three days after the operation.

Histologic examination revealed the same type of tumor as was observed at the second operation.

Necropsy.—The necropsy was performed four and one-half hours after death. The body was well developed but poorly nourished. The hair on the head had been recently shaven, and there was an irregular-shaped scar in the right parietal region, overlying a large bony defect in the calvarium. There was an old surgical scar in the lower middle region of the abdomen, extending from the symphysis pubis to the umbilicus. The gastrointestinal tract, pancreas, heart, adrenals, bladder, aorta, liver and spleen were grossly not remarkable. The significant observations were as follows:

The left pleural cavity contained 800 cc. of blood-tinged fluid. On the posterior surface of the left lung there were several firm, slightly raised tumor nodules. Sections of the lungs revealed many firm grayish white tumor nodules scattered throughout all the lobes. The largest nodule measured 2 cm. in diameter. In many places the neoplasm surrounded the bronchi and the larger blood vessels, making them unusually prominent (fig. 1C). The tracheobronchial lymph nodes contained moderately firm grayish white tumor tissue.

The abdominal cavity contained 500 cc. of clear amber fluid. The peritoneal surfaces were smooth, moist and glistening. The uterus, fallopian tubes and ovaries were absent. Several peripancreatic lymph nodes were enlarged and on section revealed firm grayish white tumor tissue. Similar tumor tissue was noted in several retroperitoneal lymph nodes.

Sections through several lumbar vertebrae disclosed one grayish white nodule of tumor measuring 1 cm. in diameter.

The brain was removed with some difficulty. Many fibrous adhesions were observed between the dura mater and the brain in the region of the old craniotomy

wounds in the right parietal region. There was a mass of firm grayish white tumor tissue, measuring 4 cm. in diameter, beneath the scar in the dura mater. The tumor displaced the anterior part of the occipital lobe and extended medially to involve the corpus callosum, but nowhere was there invasion of the brain tissue.

Histologic examination of the tumor from the brain, the lungs, the tracheo-bronchial, periaortic and peripancreatic lymph nodes and the lumbar vertebra revealed essentially the same type of tumor as was observed in the previously described operative specimens.

In the sections from the lung there was a striking tendency for the cells to grow along the interlobular septums and to infiltrate the perivascular and peribronchial lymphatics.

Pathologic Diagnoses.—These were malignant arachnoidal fibroblastoma involving the parietal and occipital lobes of the brain; metastatic arachnoidal fibroblastoma of the lungs, the pleura, tracheopulmonary lymph nodes, peripancreatic lymph nodes and a lumbar vertebra; ascites; surgical absence of the uterus, fallopian tubes and ovaries.

The tumor removed at the first operation in this case was fairly typical arachnoidal fibroblastoma, and it was only because of numerous mitotic figures that a guarded prognosis was given. The tumor observed from the subsequent operations and at necropsy was slightly more anaplastic and less differentiated, but there can be no reasonable doubt that this was the same type of tumor, which strikingly resembled arachnoidal fibroblastoma. The observation at necropsy of widely spread tumor in the lungs, peribronchial and abdominal lymph nodes and a lumbar vertebra raises the question whether the tumor within the calvarium was the primary one or merely a metastasis from some other focus of tumor. We feel that the possibility of the intracranial tumor being a metastasis can be eliminated for the following reasons:

The tumor involving the lungs, the lymph nodes and the bone marrow showed the same growth characteristics and the same histologic types of cells as the intracranial tumor, which was fairly typical of arachnoidal fibroblastoma. The tumor from the lung showed the same characteristic flattened endothelial-like cells arranged in sheets. As previously pointed out, this tumor in all instances produced significant amounts of collagen and reticulum, which definitely established the fibroblastic nature of the growth. Furthermore, we know of no fibroblastic tumor of extracranial origin that possesses the characteristic cells and arrangement of cells of arachnoidal fibroblastoma which might be confused.

Further indication that the brain lesion was the primary locus is the fact that this patient's first symptom of disease was referable to her head, and this occurred three and a half years before her death.

The operation for removal of the patient's pelvic organs a year and a half after the development of intracranial disease would seem not to have any relation to the question of extracranial primary tumor. We were not able to obtain the pathologic specimen of the uterus, but the patient's history was typical of pelvic inflammatory disease and a report

from the hospital where the pelvic operation was performed stated that this was the pathologic diagnosis and that there was no tumor. Furthermore, there is no reason to believe that this type of tumor could be primary in pelvic organs.

From the evidence presented we feel justified in concluding that this tumor was primary in the meninges and that the disseminated growths in the body were metastatic. This tumor is regarded as an example of a malignant type of arachnoidal fibroblastoma which metastasized into distant organs.

CASE 2.—Fibrosarcoma of the leptomeninges, followed thirteen years later by metastases to the liver, the left lung and the mediastinum.

History.—C. S., a married woman aged 38, entered the Barnes Hospital Jan. 31, 1928. About eighteen months before, her husband noticed that she was unusually emotional and irritable. In June 1927 it was first noticed that she had difficulty in talking, frequently used wrong words and had what was described as partial nominal aphasia. In October 1927 intense headaches first developed, with associated attacks of vomiting and some diplopia. Early in January 1928 the patient had an attack of unconsciousness and remained stuporous for twenty-four hours, with complete paralysis of the right arm and the right leg. This paralysis had improved somewhat at the time of entry into the hospital.

At the time of admission the patient had choked disks with exudate and hemorrhages in the fundi. There was partial right homonymous hemianopia. The Babinski and Oppenheim signs were positive on the right with well sustained ankle clonus. The patient showed nominal aphasia and marked emotionalism with constant crying.

The diagnosis of tumor of the left temporal lobe was made, and on February 6 the patient was operated on. At operation the ventricles were tapped and found collapsed. A needle inserted through the posterior part of the temporal lobe on the left side encountered a solid tumor. The incision was then extended posteriorly and the bone rongeuired away in order to obtain adequate exposure of the tumor, as it lay far back in the temporal lobe. The tumor was dissected out without further difficulty. It was well encapsulated and shelled out easily from the brain, which it had displaced, and weighed 60 Gm. (fig. 2A). Externally it was covered by a well defined fibrous capsule. Cut section revealed a firm gray white tissue.

Histologic examination of the tumor stained with hematoxylin and eosin revealed a highly cellular tumor tissue. The individual cells were elongated and spindle shaped and resembled fibroblasts. The cells were arranged in broad parallel sweeping formations (fig. 2B). In some areas narrow bands of cells were noted to intertwine, giving a woven appearance to that particular area of the tumor. There was a slight tendency for palisading of the nuclei in several places. Mitotic figures were frequently observed. A section stained by Mallory's phosphotungstic acid-hematoxylin method revealed a moderate amount of fine brownish red collagen between the tumor cells and fine blue-staining intracellular fibrils. The pathologic diagnosis was fibrosarcoma of the leptomeninges. However, in 1928 the diagnosis of meningioma was given, and the fact that the patient subsequently remained well for thirteen years supported that diagnosis.

The patient made an uneventful recovery and was discharged from the hospital February 19. At the time of discharge the papilledema had improved markedly. There remained only slight speech disturbance.

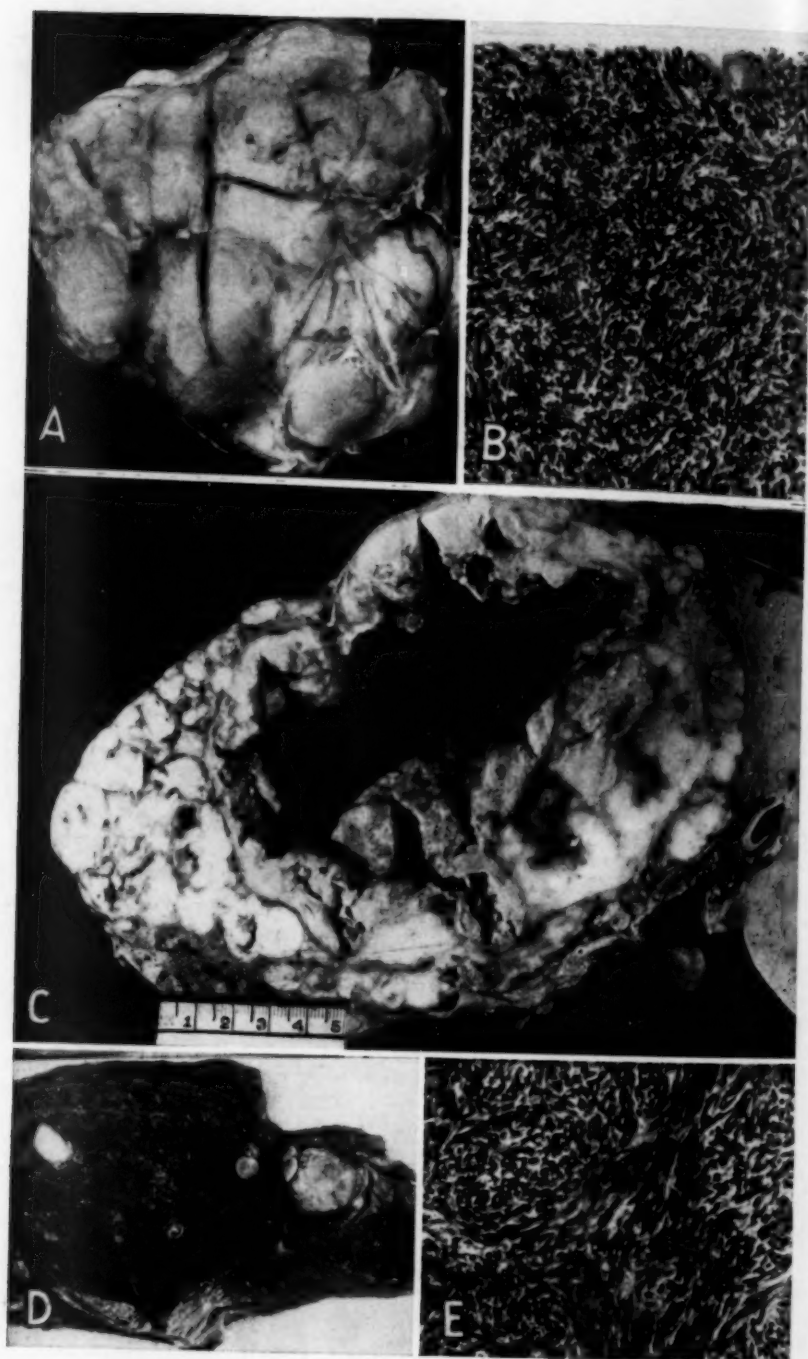


Figure 2

(See legend on opposite page)

Periodic check-ups showed that the patient had completely recovered. Ten years after her operation, on the anniversary of her operation, she returned for a check-up to show how thoroughly and completely she had recovered from her disease.

In March 1941, thirteen years after her operation she was admitted to the Missouri Baptist Hospital as the patient of Dr. Cleveland H. Shutt for a tumor of the liver. Laparotomy revealed the surface of the liver covered with grayish white nodules of tumor. After biopsy of the tumor a section was sent us. The pathologic diagnosis of the tumor was fibrosarcoma.

The patient did poorly and died in June 1941. Through the cooperation of Dr. Shutt we obtained permission for a complete necropsy.

Necropsy.—Necropsy was performed three hours after death. The body was well developed and well nourished. There was soft pitting edema of the subcutaneous tissues of the lower extremities and a moderate amount of edema of both eyelids. The abdomen was distended, and there was a linear white scar in the upper right quadrant. An old healed surgical scar was noted in the left parieto-temporal region, which covered a bony defect in the calvarium. The heart, spleen, pancreas, adrenals, kidneys, bone marrow, reproductive organs and gastrointestinal tract were grossly not remarkable. The significant observations were as follows:

The peritoneal cavity contained approximately 3,000 cc. of clear amber fluid. The peritoneal surfaces were free, smooth and glistening. Each pleural cavity contained approximately 400 cc. of clear amber fluid. A 3 by 5 cm. tumor mass was noted in the lower mediastinum between the esophagus and the apex of the heart. The tumor was well circumscribed and on section revealed a firm grayish-white tissue.

The liver was approximately six times normal size, weighing 8,900 Gm. The enlargement was most marked in the right lobe, where nearly the entire parenchyma was replaced by grayish white tumor tissue which in many places produced raised nodules on the surface of the organ. Less frequent and smaller tumor nodules were observed on the surface of the left lobe of the liver. On section, nearly the entire right lobe was replaced by a round, well circumscribed, moderately firm, grayish white mass of tumor tissue measuring 15 cm. in diameter (fig. 2 C). The center of the tumor mass was necrotic and liquefied. Surrounding the large tumor mass were innumerable smaller satellite nodules, the largest measuring 3 cm. in diameter. The left lobe contained scattered nodules of the same type of tumor, the largest measuring 3 cm. in diameter.

EXPLANATION OF FIGURE 2 (CASE 2)

A, operative specimen weighing 60 Gm., showing an irregularly lobular surface. The specimen measured 5.5 by 6 by 5 cm.

B, spindle-shaped fibroblastic cells with parallel arrangements forming intertwining bundles. Hematoxylin and eosin stain; $\times 140$.

C, transverse section through the liver. There is almost complete replacement of the left lobe by tumor. The center of the large tumor mass is liquefied.

D, section of the lower lobe of the left lung showing nodules of firm gray-white metastatic tumor.

E, general structure of the tumor. Note the spindle-shaped cells with parallel arrangements and a marked tendency to form intertwining bundles. Hematoxylin and eosin stain; $\times 140$.

Section of the left lung revealed three discrete, well circumscribed, firm, grayish white nodules of tumor in the lower lobe, measuring 2, 1 and 0.5 cm. in diameter, respectively (fig. 2D). The tumor nodules were similar grossly in all details to the tumor observed in the liver and the mediastinum. No tumor was noted in the right lung.

Removal of the calvarium revealed many strong fibrous adhesions between the dura mater and the pia-arachnoid beneath the bony defect in the left parietotemporal region. No tumor was found within the calvarium.

Histologic examination of the tumor specimens from the liver, the mediastinum and the lung revealed essentially the same type of tumor in each location. The tumor in all instances was remarkably similar to the tumor previously described, removed from the brain thirteen years before. However, in all of the sections of tumor removed at necropsy there was a slightly greater tendency for the cells to arrange themselves in narrow bundles and form many tight interwoven formations (fig. 2E). Mitotic figures were frequently observed.

Sections stained by Mallory's phosphotungstic acid-hematoxylin method revealed a moderate amount of fine brownish red collagen between the cells and extremely fine blue-staining intracellular fibrils. Perdrau preparations for reticulum revealed fine wavy fibrils of reticulum between the individual tumor cells.

Pathologic Diagnoses.—These were: healed wound from craniotomy in the left parietotemporal area (history of removal of fibrosarcoma thirteen years before); metastatic fibrosarcoma of the liver, the mediastinum and the lower lobe of the left lung; ascites (3,000 cc.); bilateral hydrothorax.

This is a most remarkable case from many standpoints. That this patient was cured of her intracranial disease and remained symptom free for twelve years only to succumb to an intra-abdominal tumor is a fact of unusual interest. The relationship of the brain tumor to the abdominal tumor raises several possibilities. First, did the tumor in the abdomen represent a metastasis from the intracranial tumor? Second, did the intracranial tumor which was cured by operation represent a metastasis from the tumor in the liver which killed the patient thirteen years later, or, third, did the two tumors bear no relationship to one another, being merely tumors of multicentric origins?

We believe that the bulk of the evidence favors the first assumption, namely, that the intracranial lesion was primary and the tumor in the liver a metastasis. In favor of this opinion is the fact that the histologic type of tumor in each location was identical and that this tumor was histologically characteristic fibrosarcoma. Fibroblastic tumor of the leptomeninges though extraordinarily rare, as the listed cases from the literature would indicate, can give rise to metastases. Probably most important is the interval of time between the appearance of the two tumors. It would seem most reasonable that if the tumor in the liver had been the primary one and the tumor in the brain a metastasis, the patient should have presented symptoms of hepatic disease possibly before or shortly after the appearance of her intracranial disease but

certainly not after thirteen years. There can be little question that the tumor from the liver and the intracranial tumor represent the same growth because of their histologic similarity. The most reasonable sequence of events was that the completely removed tumor of the leptomeninges had already given rise to a metastasis to the liver which grew slowly and gave clinical symptoms twelve years later.

We regard this tumor as an instance of fibrosarcoma arising from the leptomeninges, having the same cell of origin as arachnoidal fibroblastoma and metastasizing to the lung, the mediastinum and the liver.

CASE 3.—Fibrosarcoma of the leptomeninges in a 12 year old girl with metastasis to the pleura.

History.—N. L., a girl aged 12 years, entered the St. Louis Children's Hospital Sept. 6, 1939. Her history was noncontributive except that for the past five years she had suffered from headaches and attacks of vomiting. She had had a number of convulsions that were not accompanied by loss of consciousness. The convulsions had become more frequent during the weeks before admission.

The positive neurologic findings were: choked disks with marked tortuosity of the vessels; positive Macewen's sign; lateral nystagmus to the right; generalized muscular weakness. A cerebellar tumor was suspected, and a ventriculographic study was undertaken. When the needle was inserted into the right parieto-occipital region, it apparently entered the tumor, as there was a severe hemorrhage. In order to control the bleeding, the bone was quickly rongeured away so that sufficient tissue could be removed to control the hemorrhage. At that time it was not clear whether there was a tumor or a subdural hematoma; therefore an occipital bone flap was turned down. A subcortical tumor was exposed, which was soft and which was removed by suction. The tumor was very vascular and bled so freely that bits of muscle had to be used to control the bleeding. Because of this profuse bleeding, it was impossible to remove the tumor completely. The tumor tissue removed weighed 106 Gm. and on cut section was a moderately firm grayish red tissue. The postoperative course was uneventful except for a number of transfusions, and the patient was discharged on September 28.

Histologic examination of several pieces of the tumor revealed a moderately cellular tumor tissue. The different pieces revealed two markedly different types of growth. In some parts the tumor was composed of tightly packed spindle-shaped cells with small elongated nuclei arranged in broad parallel sweeping formations (fig. 3A). Finely distributed fibers of reticulum were observed in the Perdrau preparation in those areas of the tumor having the elongated cells. Other pieces of the tumor revealed less differentiated cells. In these areas the cells were round or slightly elongated with plump vesicular chromatic nuclei. The cells demonstrated a marked tendency to grow in closely packed groups, particularly around blood vessels. Frequent mitotic figures and an occasional tumor giant cell were noted in both types of growth. Transitional types of growth of the tumor were observed between the type containing the spindle-shaped cells and the type with the poorly differentiated plump cells.

A section stained by Mallory's phosphotungstic acid-hematoxylin method revealed much brownish red intercellular collagen between the spindle-shaped tumor cells. Fine blue-staining intracellular fibrils were also noted in these

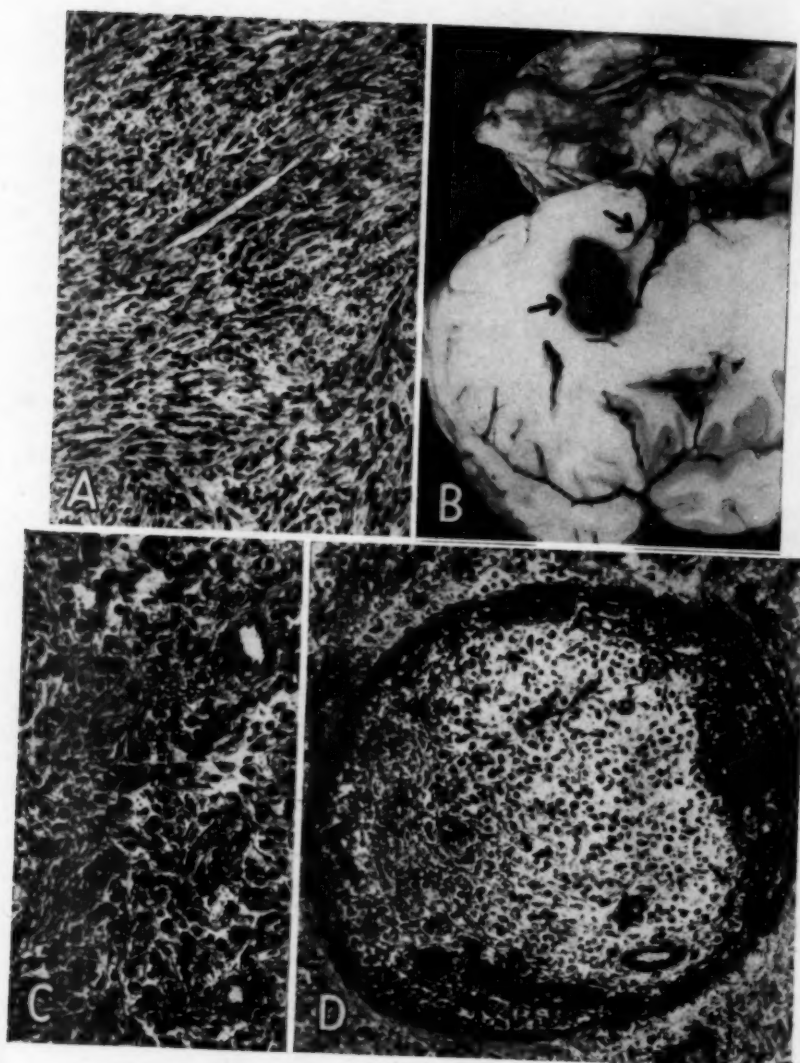


Figure 3

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cells. In the areas of poorly differentiated tumor with the oval-shaped cells no intercellular material could be made out nor did the cells contain intracellular fibrils. Throughout the poorly differentiated tumor were scattered areas of necrosis and in some instances small areas of hemorrhage. The pathologic diagnosis was fibrosarcoma of the leptomeninges.

Two months after discharge the patient was readmitted because of continued headaches and convulsive movements of the right arm.

Craniotomy was performed a second time, and on opening the old wound a large recurrent tumor was noted. At this operation a well defined line of cleavage was observed between the brain and the tumor. It was believed that a nearly complete removal was carried out. There was, however, some question of tumor remaining attached to the falx. The tumor removed weighed 29 Gm. The patient made an uneventful recovery and was discharged on November 27.

Histologic examination of the tumor removed at the second operation showed many large pieces of dense collagenous tissue with only a few small islands of tumor cells. The tumor cells were of the poorly differentiated type observed in the specimen from the first operation. There were frequent mitotic figures, and no appreciable intercellular collagen could be observed in sections stained by Mallory's phosphotungstic acid-hematoxylin method. The pathologic diagnosis was "poorly differentiated fibrosarcoma."

The patient was readmitted Jan. 10, 1940 with symptoms suggestive of a recurrence of the tumor. Because the tumor was known to be so rapidly growing, it was decided to treat the patient with high voltage roentgen rays, as operation had offered no relief. In spite of the roentgen treatment the patient's symptoms became worse, and one month later she became stuporous. She remained in a stuporous condition for over two months and finally died May 29, 1940.

Necropsy.—Necropsy was performed ten hours after death. The body was well developed but poorly nourished. There was an old healed left occipitoparietal craniotomy wound. The heart, liver, spleen, pancreas, adrenals, kidneys, reproductive organs, bone marrow and gastrointestinal tract were grossly not remarkable. The significant observations were as follows:

EXPLANATION OF FIGURE 3

Fig. 3.—*A* (case 3), section of tumor removed at the first operation. Note the spindle-shaped cells resembling fibroblasts arranged in sweeping formations and showing a striking tendency to form individual bundles that interlace. Hematoxylin and eosin stain; $\times 140$.

B (case 3), coronal section through the posterior part of the frontal lobes with a piece of overlying dura mater attached. The dura mater is nodular and greatly thickened by tumor. The upper arrow indicates the falx cerebri, which is also heavily infiltrated with tumor. The lower arrow indicates a large nodule of tumor with hemorrhage displacing the temporal lobe.

C (case 4), area of tumor showing somewhat elongated cells with prominent vesicular nuclei. The fine black lines between the cells are collagen fibers, which are most conspicuous in those places showing the more elongated cells. Mallory's phosphotungstic acid-hematoxylin stain; $\times 140$.

D (case 4), small vein completely filled with anaplastic tumor cells. Note the small capillaries in the lower part of the vein attempting to recanalize the vessel. Mallory's phosphotungstic acid-hematoxylin stain; $\times 85$.

The brain was removed with considerable difficulty because the dura mater in the left frontal and parietal regions was markedly thickened by a firm grayish white tumor tissue invading its inner surface, which was adherent to the brain in many places. There were numerous discrete nodules of firm tumor attached to the inner surface of the dura mater, in the left middle fossa and the left side of the falx cerebri (fig. 3B). The tumor nodules ranged from 5 to 25 mm. in diameter. The thickened nodular dura had produced depressions in the underlying brain tissue. Two irregularly outlined defects were noted, one in the left occipital lobe, 6 cm. in diameter, and one in the left frontal lobe, 7 cm. in diameter. The cerebral convolutions were flattened, and the sulci were obliterated. Step sections through the brain revealed marked enlargement of the left cerebral hemisphere with deviation of the septum pellucidum to the right. Beneath the defect previously mentioned in the left frontal lobe, there was a well defined, granular, red, yellow and brown mass of tumor measuring 6 cm. in diameter and extending 7 cm. posteriorly from the anterior part of the left frontal lobe. The anterior horns of the left lateral ventricles were completely filled with firm tumor tissue showing many areas of blackish red hemorrhage. A mass of similar tumor tissue was found in the right frontal lobe attached to the falx cerebri, measuring 2 cm. in its greatest diameter. The falx was infiltrated with tumor and approximately four times normal thickness. The left occipital lobe and the posterior part of the left parietal lobe were replaced by tumor tissue similar in type to that noted in the frontal lobes.

On the visceral pleura of the lower lobe of the right lung near the interlobar fissure a firm grayish white plaque of tissue measuring 7 mm. in diameter was observed. Section of the plaque revealed a moderately firm, grayish white tissue that extended about 3 mm. into the lung.

Three sections taken from the intracranial tumor revealed the same type of neoplasm as was observed in the previously described surgical specimens. A section of the small nodule in the lower lobe of the right lung revealed a small area of tumor beneath the pleura. The tumor cells were elongated and spindle shaped, with deep staining small hyperchromatic nuclei. In some areas there was considerable pleomorphism of the cells, and mitotic figures were observed. The tumor cells had infiltrated along the interlobular septums and were found in several peribronchial lymphatics.

Pathologic Diagnoses.—These were: fibrosarcoma of the leptomeninges involving the frontal lobes, the left parietal and occipital lobes, the falx cerebri and the dura mater; metastatic fibrosarcoma of the pleura of the lower lobe of the right lung; partially healed wounds from craniotomy in the left occipital area.

The tumor in this case was poorly differentiated fibrosarcoma showing many areas of anaplasia. There can be no question about this tumor being fibrosarcoma, because the cells in many areas resembled fibroblasts and were producing collagen and reticulum. The most unusual feature of the case was the small nodule of tumor observed beneath the pleura of the right lung. Though this nodule was small, it was definitely neoplastic and histologically resembled the intracranial tumor. The cells were elongated, resembling fibroblasts, and were producing collagen and reticulum. There were mitotic figures present.

It is unusual to find a tumor belonging to the arachnoidal fibroblastoma group in so young a patient. In Cushing and Eisenhardt's^{3d} entire series of 313 cases there were only 6 patients at or below 15 years of age. Nevertheless, there is good reason for believing this tumor is related to the arachnoidal fibroblastoma group because it was in a parasagittal position and its growth characteristics were similar to those of arachnoidal fibroblastoma, which strongly indicates the arachnoid as the site of origin.

We believe this tumor to be a primary tumor of the leptomeninges with a metastasis in the lung.

CASE 4.—An extremely anaplastic and rapidly growing fibroblastic tumor arising from the leptomeninges.

History.—A. G., a man aged 33, was admitted to the Barnes Hospital Sept. 28, 1940. For two years prior to entry he had noticed periodic occipital headaches, which had markedly increased in severity and had been accompanied by nausea and vomiting in the six weeks before his admission to the hospital. The headaches had become so disturbing that for relief huge doses of acetylsalicylic acid, up to 50 grains (3.23 Gm.) a day, were taken by the patient.

Neurologic examination disclosed choked disks and slight flattening of the left side of the face. The visual fields and all reflexes were normal. There were so few positive findings that a ventriculogram had to be made. This revealed a tumor in the right temporal lobe, and craniotomy was immediately performed. When a needle was inserted into the right temporal lobe, marked resistance was encountered at a depth of approximately 4 cm. To expose the tumor, the cortex of the temporal lobe had to be resected, and a completely encapsulated, grayish pink tumor mass was exposed. The tumor was removed by first sucking out the contents and then collapsing the capsule. It was observed that the capsule of the tumor was attached to the upper surface of the tentorium just lateral to the incisura. After the capsule had been removed, the vessels of the circle of Willis were plainly visible. The tumor weighed 16 Gm. The patient's wound healed without difficulty but a postoperative psychosis developed, which subsided after a few days. At the time of discharge, October 25, he was well oriented, although somewhat talkative and noisy.

Histologic examination of the specimen stained by the phloxine-methylene blue technic revealed a highly cellular tumor tissue. The cells were round to oval in most areas and contained vesicular, moderately chromatic nuclei. In some areas, however, the tumor cells showed a tendency to elongate into cells resembling fibroblasts. An occasional tumor giant cell was found, and mitotic figures were frequently observed. No characteristic arrangement of the cells was discernible. For the most part, they were closely packed together in moderately large collections and were separated by broad bands of connective tissue stroma. Several large areas of the section showed necrosis of the tumor and its stroma. A section stained by Mallory's phosphotungstic acid-hematoxylin method revealed a few fine, deep blue-staining intracellular fibrils in the tumor cells showing a tendency to elongate and a small amount of brownish red-staining intercellular collagen (fig. 3 C). Intercellular collagen and intracellular fibrils were absent in the remaining cells, showing no tendency to differentiate into fibroblasts. The pathologic diagnosis was rapidly growing anaplastic fibrosarcoma.

The patient was readmitted December 4 because of a recurrence of his original symptoms of headache and vomiting. At this time there was marked personality change. The neurologic examination showed choked disks, paralysis of the left side of the face and pathologic reflexes on the left side of the body, and homonymous hemianopia could be demonstrated by confrontation.

Following the making of a ventriculogram which revealed a markedly dilated ventricle on the left side, the patient was reoperated on December 7. At the old operative site, a recurrence of the tumor was noted. The neoplasm had grown down into the posterior fossa through the incisura. During removal of the tumor a large vessel was injured in the region of the circle of Willis, which had to be clipped. For this reason, a complete removal of the tumor was not attempted. The tumor removed weighed 23 Gm.

The patient never regained consciousness and died the night following the operation.

The tissue removed at the second operation and stained with hematoxylin and eosin showed essentially the same type of growth as was observed in the tumor removed at the first operation. The connective tissue stroma, however, appeared slightly more abundant, and in those areas of the tumor showing slightly elongated cells there was observed in the phosphotungstic acid-hematoxylin stain fine, reddish brown-staining collagen fibers between the individual cells. Generally, however, the tumor cells appeared more anaplastic than those in the tumor removed at the first operation. Pleomorphism was marked, and mitotic figures were abundant. A moderate-sized vein in the section was completely filled with tumor cells (fig. 3D). Also greater vascularity was noted in this specimen than in the one removed at the first operation.

Necropsy.—This was performed one hour after death. The body was well developed and well nourished. There was a recent craniotomy wound in the right occipital region. The heart, lungs, liver, spleen, pancreas, adrenals, kidneys, gastrointestinal tract and reproductive organs were grossly not remarkable. The significant observations were as follows:

The brain was removed with some difficulty, and there was a moderate amount of freshly clotted blood beneath the dura mater in the region of the craniotomy wound in the right temporal lobe. There was an operative defect in the right temporal lobe, measuring 3 cm. in diameter and extending 1 cm. into the brain. The subarachnoidal space over the cerebral hemispheres and the basilar structures contained a moderate amount of clotted brown-red blood. Attached to the tentorium and to a small piece of dura mater in the right temporal region were two moderately firm pieces of grayish white tumor tissue, the largest measuring 1 cm. in diameter. Step sections through the brain revealed focal areas of hemorrhage in the tissue surrounding the operative defect and a moderate amount of brownish red clotted blood in the ventricles.

The lower lobe of the left lung contained several foci of brownish gray consolidation.

Histologic examination of the tumor nodules attached to the dura mater and the falx revealed the same type of tumor as was described in the surgical specimens.

Pathologic Diagnoses.—These were: fibrosarcoma of the right side of the tentorium, involving the right temporal lobe of the brain; recent wound of a right temporal craniotomy; moderate subarachnoidal hemorrhage; bronchopneumonia of the lower lobe of the left lung.

The cells in this tumor were so extremely anaplastic that the diagnosis of a fibroblastic tumor was made with considerable difficulty. For

the most part, the cells showed no differentiation, and it was only in a few scattered areas that elongated cells resembling fibroblasts producing intercellular collagen and reticulum were observed. It was these selected areas, together with the fact that the tumor was sharply demarcated from the brain and in no instance invading it, that convinced us this tumor was primary in the leptomeninges.

The histologic diagnosis of this tumor should be fibrosarcoma, because of the fibroblastic character of the growth which was obviously a rapidly growing malignant tumor.

It seems somewhat paradoxical that in this case, in which the most rapidly growing anaplastic tumor in the series was noted, metastases did not occur, while metastases were observed in the 3 previous cases. However, the invasion of blood vessels by the tumor shown in figure 3D would clearly indicate that had the patient lived long enough metastases would most probably have occurred. Still another possibility is that metastases did occur but were of microscopic size and escaped the routine postmortem examination.

COMMENT

The four tumors in this study were remarkably dissimilar in several respects, yet we believe they should be grouped together because in our opinion they have the same cell of origin and represent merely extreme variations of the same histologic type of tumor. Comment on these cases, therefore, will be concerned with the following four points: (1) the reason why we regard these tumors as a single histologic type, (2) the relationship of these tumors to the benign arachnoidal fibroblastoma, which we believe has the same cell of origin, and (3) the selection of a name for this type of tumor that would be suitable and accurate.

Each tumor in this series has exhibited sufficiently definite histologic characteristics to establish the type of growth as of mesodermal origin. The cells in at least some parts of every tumor were elongated, somewhat spindle-shaped cells resembling fibroblasts, which contained characteristic fibroglia fibrils and were producing collagen and reticulum. It should be noted that the cases have been arranged in a sequence to indicate the graded degrees of differentiation of the tumor cells. The most differentiated type of tumor was that in case 1, while the tumor in case 4 was the most anaplastic and poorly differentiated of the group.

To recapitulate briefly for the sake of emphasis, case 1 showed the highest degree of differentiation, with the cells closely resembling the type cell of arachnoidal fibroblastoma both in the intracranial tumor and in the visceral metastases. The tumor in this case might well be called a malignant type of arachnoidal fibroblastoma.

In case 2 the cells showed no resemblance in any of the sections to the type cell of arachnoidal fibroblastoma. The intracranial tumor and the visceral metastases observed thirteen years after the removal of the intracranial tumor were highly characteristic fibrosarcoma and were indistinguishable from the usual type of fibrosarcoma occurring in other locations in the body. The individual cells demonstrated a high degree of differentiation and were producing large amounts of collagen and reticulum. Objectively, this tumor was well differentiated fibrosarcoma, whose individual cells showed none of the morphologic characteristics of arachnoidal fibroblastoma cells. However, the primary tumor involving the brain showed the same local growth characteristics as arachnoidal fibroblastoma, being a well encapsulated tumor attached to the meninges and not invading the brain. For this reason, we believe this tumor arose from the cells of the arachnoid and therefore has a common cell of origin with arachnoidal fibroblastoma. This case, then, is to be regarded as an instance of typical and characteristic fibrosarcoma arising from arachnoidal cells.

The tumor in case 3 exhibited still less differentiation of its cells than the tumor observed in case 2. In scattered areas the tumor cells showed an extreme degree of anaplasia and were producing no appreciable intercellular collagen or reticulum, yet for the most part the tumor was highly characteristic, rapidly growing, rather poorly differentiated fibrosarcoma. We believe this tumor arose from arachnoidal cells and therefore has a common cell of origin with the fibroblastoma group because, like the primary tumor in case 2, it did not invade the brain but was a well encapsulated, circumscribed tumor attached to the meninges in a parasagittal position.

The most poorly differentiated and anaplastic tumor was in case 4. As has been mentioned, it was only after a most careful and searching investigation that this tumor was identified to our satisfaction as a fibroblastic tumor, for only in small scattered areas did the cells show any differentiation into cells resembling fibroblasts. Generally the cells showed no characteristic form or arrangement and produced no intercellular material. Like the other tumors in this study, position and local growth behavior strongly indicate that the tumor arose from arachnoidal cells and would therefore have a common cell of origin with arachnoidal fibroblastoma.

The foregoing short histologic résumé of the 4 cases adequately illustrates our first premise, namely, that the histologic character of all these tumors was that of a fibroblastic or a mesodermal tumor, the individual tumors differing only in the degree of anaplasia and differentiation of their cells from most primitive anaplastic sarcoma (case 4), on the one hand, to fairly well differentiated fibroblastic tumor closely resembling arachnoidal fibroblastoma (case 1), on the other hand

Furthermore, the four primary intracranial tumors showed that they were remarkably similar in their growth behavior, for in no instance did the tumor invade the brain but always remained sharply demarcated and circumscribed. Furthermore, all of the tumors occurred in a parasagittal position, which is the most frequent site of origin for arachnoidal fibroblastoma. These observations clearly indicate that these tumors were not primary in the brain but arose from the adjacent meninges, which we feel is strong evidence that they are of a single histogenetic type.

Because of the highly controversial question of the histogenesis of the cell type of arachnoidal fibroblastoma the following point of discussion considering the relationship between arachnoidal fibroblastoma and the tumors reported in this study is undertaken with great temerity and utmost caution. There is no dissenting opinion in the literature regarding the cell of origin of arachnoidal fibroblastoma but it is the histogenesis of that cell which is controversial. Briefly, the flattened elongated fibroblastic-appearing cell of the leptomeninges forming the arachnoidal villi is universally accepted as the cell of origin. One group of investigators¹² has expressed the belief that this cell is a highly specialized fibroblastic cell derived from the mesenchyme forming the leptomeninges, while the other group¹³ has asserted that the leptomeninges, or at least the arachnoidal cells of the leptomeninges, are derived from neural crest cells and therefore are not of mesodermal origin. It is freely admitted by both groups, however, that these tumors, no matter what the cell of origin is, behave like fibroblastic tumors: the cells morphologically resemble fibroblasts; they produce collagen and reticulum and have characteristic intracellular fibrils.

The tumors in this study are to be differentiated from the arachnoidal fibroblastoma group because each tumor on histologic examination was definitely a malignant tumor and metastases occurred in 3 of the cases. Even though in case 1, in which the cells in arrangement and in cytologic detail closely resembled those of arachnoidal fibroblastoma, the diagnosis of the tumor should rightfully be fibrosarcoma because of the number of mitotic figures present, the fact that metastases occurred and the unmistakable fibroblastic nature of the growth. In the remaining 3 tumors the arrangement of the cells and the cytologic detail in no way resembled those of the type cells in arachnoidal fibroblastoma. However, these tumors fulfilled all the accepted criteria for a malignant tumor and, as has been clearly pointed out, apparently arose from arachnoidal cells. These tumors, then, represent malignant transforma-

12. Mallory, F. B.: *J. M. Research* **41**:349, 1920. Penfield, W.: *Surg., Gynec. & Obst.* **45**:178, 1927.

13. Oberling, C.: *Bull. Assoc. franç. p. l'étude du cancer* **11**:365, 1922. Roussey, G., and Cornil, L.: *Ann. d'anat. path.* **2**:63, 1925.

tion of the arachnoidal cell, which is admittedly the cell of origin of arachnoidal fibroblastoma. We feel, therefore, that the fact such definitely characteristic fibrosarcoma was primary in the arachnoid is strong evidence that this structure is of mesodermal rather than neuroectodermal origin. Objectively stated, malignant neoplasia or arachnoidal cells produce tumors indistinguishable from malignant fibroblastic tumors arising from tissues known to be of mesodermal origin in other locations in the body. Further support for this conclusion is found in the recently reported production of brain tumors with carcinogenic agents in experimental animals.

Several investigators¹⁴ have reported the successful production of glioma with methylcholanthrene and in addition observed in such studies the occurrence of highly characteristic fibrosarcoma in a significant number of cases (26 per cent in Zimmerman's series). The tumors diagnosed as fibrosarcoma were apparently produced by the action of the carcinogenic agent on the leptomeninges, again demonstrating that malignant neoplasia of arachnoidal cells results in fibrosarcoma.

To summarize, then, we regard the four tumors of our series as malignant tumors of arachnoidal origin which have exhibited many of the characteristics of malignant fibroblastic tumors. The fact that they showed such profound variation in their type of growth and degree of differentiation toward fibroblastic cells is not against the diagnosis of fibrosarcoma since fibrosarcoma frequently shows extreme anaplasia of its cells so that differentiation of such fibrosarcoma from poorly differentiated carcinoma is at times difficult. The very fact that there was great difference in the degree of differentiation of the several tumors in our series might be cited as an additional point for the accuracy of the diagnosis of fibrosarcoma.

Whether these tumors represent malignant transformation of previously existing benign arachnoidal fibroblastoma or are malignant tumors that have arisen directly from the arachnoidal cells is an academic question and cannot be answered with certainty. No evidence was observed in any of the tumors of a previously benign type of growth, although it is entirely possible that if an original benign focus did exist it might have been completely overlooked or destroyed by the more rapidly growing malignant tissue.

There can be no doubt but that the tumors reported here are mesoblastic in nature and deserving of the term "sarcoma." Furthermore, the prefixed term "fibrosarcoma" can be accurately applied to these tumors, for even in the most poorly differentiated one the cells in some

14. Seligman, A. M., and Shear, M. J.: *Am. J. Cancer* **37**:364, 1939. Peers, J. H.: *Am. J. Path.* **16**:799, 1940. Zimmerman, H. M., and Hildegarde, A.: *Cancer Research* **1**:919, 1941.

areas showed definite signs of differentiation into cells resembling fibroblasts. Since evidence has previously been presented that these tumors represent neoplasia of arachnoidal cells, we feel the word "arachnoidal" should preface the term "fibrosarcoma." The diagnosis, therefore, would be "arachnoidal fibrosarcoma." This term is accurate and appropriately applied, for it is descriptive of the place of origin and the cell of origin of these tumors. The use of the term "osteogenic sarcoma," now generally accepted for the manifold primary tumors of bone, is predicated on the same type of reasoning.

SUMMARY

Careful review of the literature revealed only 4 cases of intracranial tumor of arachnoidal origin which had metastasized into distant organs. This paper reports 4 additional cases of malignant intracranial tumor believed to be primary from arachnoidal cells. Metastasis into distant organs occurred in 3 of these cases, and in the fourth case invasion of blood vessels by tumor strongly suggested that tumor metastases may have been present but possibly were of microscopic dimensions.

In all of the cases the nature of the growth was characteristic of a mesodermal tumor, for the cells showed varying degrees of differentiation into fibroblasts. These tumors are closely related to arachnoidal fibroblastoma and merely represent varying degrees of malignant transformation of arachnoidal cells, which are admittedly the cells of origin for benign arachnoidal fibroblastoma, for in 1 case the tumor cells closely resembled the type cell of arachnoidal fibroblastoma. In 2 cases the type of growth and the cells were typical of fibrosarcoma as observed in any other tissue in the body. We feel that these cases offer additional evidence for the mesodermal origin of the arachnoidal cell and the leptomeninges since these tumors which apparently arose from arachnoidal cells were characteristic fibrosarcoma.

The term "fibrosarcoma," designating a malignant mesodermal tumor showing a type of cell resembling a fibroblast that produces collagen and reticulum, is readily applicable to all the tumors in this study. Because these tumors arise from arachnoidal cells, the term "arachnoidal fibrosarcoma" is suggested.

CONCLUSIONS

Intracranial cancers arising from the arachnoid and having the same cell of origin as arachnoidal fibroblastoma exhibit the general histologic characteristics of fibrosarcoma arising in other tissues in the body, including metastasis into distant organs. These tumors are to be regarded as malignant forms of benign arachnoidal fibroblastoma. The term "arachnoidal fibrosarcoma" is suggested for this type of malignant fibroblastic tumor arising from arachnoidal cells.

ELASTIC PROPERTIES OF THE RABBIT AORTA IN RELATION TO AGE

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The human aorta becomes more elastic (in the commonly accepted sense) from birth to about the twenty-fifth year of life. Beyond this age there is gradual but progressive loss of elasticity. This curve of elasticity has been shown by various methods. Roy¹ and Yater and Birkeland² showed that the ability of strips of aorta to extend under tension decreases as a function of age past the second decade. Winternitz, Thomas and LeCompte³ made similar observations. Wilens⁴ studied the capacity of strips of aorta to retract after nearly maximal extension. He concluded that this capacity decreases as age advances and that it varies in degree in different areas of the vessel but diminishes with advancing age at a constant rate for a given area. The mobile parts of the aorta retained more elasticity as measured by this method than did the fixed parts. The earlier work on elastic properties of the aorta was reviewed by Hass⁵ and by Wilens.⁴ Interest in the subject has centered largely about the relation of elasticity to the development of atherosclerosis. In spite of numerous studies, a causal relation has yet to be proved, although it is clear that changes in the elastic properties of the human aorta are a measure of aging.

Krafka⁶ and Roy¹ noted differences in the elastic qualities of the aortas of different species as shown by values of Young's modulus under comparable tensions⁶ and by stress-strain curves.¹ Changes associated with aging were not studied in species other than man. Hume⁷ found that the rat aorta with advancing age from 90 days up to senility

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2. Yater, W. M., and Birkeland, I. W.: *Am. Heart J.* **5**:781, 1930.
3. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: *The Biology of Arteriosclerosis*, Springfield, Ill., Charles C. Thomas, Publisher, 1938.
4. Wilens, S. L.: *Am. J. Path.* **13**:811, 1937.
5. Hass, G. M.: *Arch. Path.* **27**:334 and 583, 1939.
6. Krafka, J., Jr.: *Am. J. Physiol.* **125**:1, 1939.
7. (a) Hume, J. F.: *Am. J. Hyg., Sect. A* **29**:11, 1939. (b) Nelbach, J. H., and Herrington, L. P.: *ibid.* **22**:661, 1941.

(600 days) becomes progressively more distensible, in contrast to the behavior of the human aorta, as shown by Winternitz,³ in which there is a decrease in distensibility. Owing to differences in method and in presentation of data, it is not possible to compare accurately Hume's values with those for the human aorta.

It is of interest to determine the relation of age to the elastic properties of the aortas of species other than the rat and man as a comparative study of aging processes in general and as an analysis of factors which might favor development of atherosclerosis in one species and not in another. This report deals with the elastic properties of the rabbit aorta at different ages, and the method of procedure allows rough comparison with the data of Wilens⁴ and of Hume.^{7a}

MATERIAL AND METHODS

The aortas were obtained from 18 normal male rabbits ranging from 6 months to 5 years of age. The age distribution is shown in table 1. All except the four youngest rabbits had been kept in this laboratory for periods up to four and one-half years, serving as controls to the experiments on tuberculosis conducted by Dr. E. L. Opie and associates.

Most of the rabbits were killed by injection of air into an ear vein. The aorta was removed a few minutes later. After the surrounding fat and fibrous tissue had been dissected away, segments (rings) 5 mm. in length were cut from the upper, middle and lower regions of the descending thoracic aorta. Care was taken to apply no tension to these segments during preparation for testing, and the lower segments were chosen so as to exclude the origins of the intercostal arteries. Measurements of the original circumference and of the thickness of the vessel wall at the three levels were made from portions immediately above and below each segment to be tested, and the two values in each instance were averaged. The circumference of the opened ring of aorta was measured to the nearest 0.1 mm. by means of a Vernier caliper. The thickness of the wall was measured with a micrometer attached in an electrical circuit so that contact of the micrometer pin with the tissue was registered on a milliamper meter. The tissues were kept moist during these measurements.

The apparatus used for testing was of the same principle as that used by Hume^{7a} and is illustrated in figure 1. The segment of aorta was mounted over two horizontal pins made of piano wire, one attached to the base and the other to the lever of a modified hydrostatic balance. The loading pan and the pin were each 11 cm. from the fulcrum. Changes in the circumference of the vessel segment were magnified exactly twice by a pointer which extended 44 cm. from the fulcrum. The pointer traced a record on a smoked drum. The segment was kept immersed in physiologic solution of sodium chloride during the test, and all tests were run at room temperature. A tare weight of 1 Gm. was present at all times.

The tests were run in the following manner: By means of a small tray weighing 5 Gm. a load was applied by hand to the segment in increments of 5 Gm. up to 100 Gm. and by 10 Gm. increments thereafter until 200 Gm. had been applied. Between increments the vessel was allowed to retract, and the amount of retraction was recorded. After each change in load the kymograph

drum was rotated a short distance to obtain a record. It was found that an equilibrium was reached in a few seconds after a change in load. Supplementary tests showed that the elastic after-action¹ was extremely small for periods up to thirty minutes and could for these purposes be disregarded. One record was made for each segment. It should be pointed out that this method gives a measure of the transverse or circumferential elastic properties only. Longitudinal elasticity and the effects of pressures applied internally were not studied.

Before each segment was loaded, a base line was traced on the kymograph record with the segment suspended on the pins and with the tare weight in place. The tare weight did not visibly stretch the segment, and for calculations this base line was taken to correspond to the original circumference as measured from

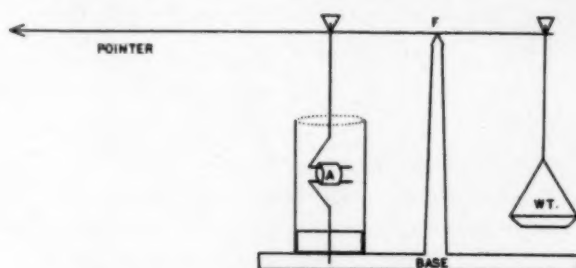


Fig. 1.—Diagram of the apparatus used to study the elastic properties of segments of the aorta. *A* is a ring of aorta suspended on pins within a vessel containing physiologic solution of sodium chloride.

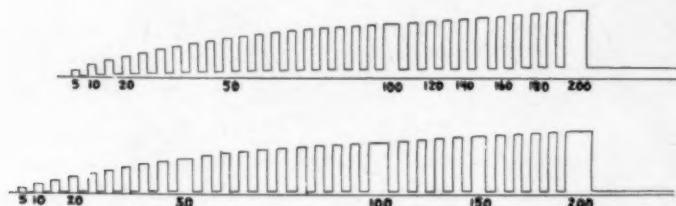


Fig. 2.—Loading records made with segments taken from corresponding levels of the aortas of a 5 year old (upper record) and a 1 year old rabbit (lower record). The circumference of the older aorta did not return so completely to its original value after stretching as did that of the younger aorta, as shown by the greater distance between the record and the base line. $\times \frac{1}{3}$.

the rings of aorta contiguous to the segment tested. The numerical values of extension and retraction as defined in the following section were calculated from measurements made directly on the records, using a ruler graduated to 0.5 mm. and a hand lens. Interpolation to within 0.1 mm. was possible, and repeated measurements of the same points differed by less than 0.2 mm., an error of less than 1 per cent. Typical loading records, made with comparable segments of aortas from a 5 year old and a 1 year old rabbit, are shown in figure 2.

The tested and the untested portions of the aortas were fixed in 4 per cent solution of formaldehyde, and microscopic cross sections were prepared. These

were stained with hematoxylin and eosin, Masson's trichrome⁸ and Weigert's stain for elastic tissue.

DEFINITIONS AND METHODS OF ANALYSIS

The term "elasticity" is used here in a descriptive sense to denote the ability of a material to stretch under tension and to return to its original form when the tension is removed.

The term "load" is used only to denote the force (weight in grams) applied as traction to the segment of vessel being tested and does not take into consideration the cross sectional area to which the force is applied. As the segments differed in cross sectional area, depending on the thickness of their walls, the load applied has relatively little significance for purposes of comparison. The term "stress" is therefore used when elastic properties are compared and is here defined as the force in grams ($\times 980$ when converted into dynes) applied per square centimeter of cross sectional area of vessel wall.

"Extension" is defined as the increase in circumference of the vessel segment beyond its original circumference when a load is applied. "Extended circumference" refers to the circumference of the vessel segment under a load producing measurable extension. "Retraction" is defined as the decrease in circumference of the vessel which occurs when the load is removed. The changes in circumference of segments are thus comparable to changes under similar stresses in the length of strips of aorta cut in a transverse direction.

Using the measurements obtained from the loading records, ratios of extension to original circumference were calculated for each of the 54 tests at loads of 20, 40, 60, 80, 100, 140 and 180 Gm. These ratios are an expression of the degrees to which the vessel segments stretch in a transverse or a circumferential direction under tension; they correspond to "tensility" as defined by Hume.^{7a} Ratios of retraction after these loads were removed to the extended circumference when these loads were applied were computed in order to compare these data with those of Wilens.⁴ During the course of the tests it was noted that the aortas of older animals did not in general retract so completely as did those of younger animals. This difference can be seen in figure 2. In order to give it numerical expression, the ratio of increase in circumference after unloading to the original circumference was calculated for each test after the loads mentioned. All ratios are expressed as percentages.

Values of these three ratios averaged for two main age groups, young and old, and for the three sites from which the segments were taken were plotted against the numerical values of stress. As a further analysis of

8. Masson, P.: J. Tech. Methods **12**:75. 1929.

the relation of extension and retraction to stress, these ratios were plotted against the logarithmic values of stress.

Values of Young's modulus for extension at loads of 20, 60, 100 and 180 Gm. were calculated from the average values of each of the five smaller age groups (table 1) and of the three groups based on the site of the segment (table 2) according to the formula:

$$M \text{ (ext)} = \frac{\text{load in Gm.} \times 980 \times \text{original circumference}}{\text{Cross sectional area in sq. cm.} \times \text{extension under load}}$$

The relation of the retraction following each unloading to the extended circumference at the load is expressed as the value of a modulus for retraction with the formula:

$$M \text{ (retr)} = \frac{\text{load in Gm.} \times 980 \times \text{extended circumference under load}}{\text{Cross sectional area in sq. cm.} \times \text{retraction after unloading}}$$

The relation of the increase in circumference with the retraction following each unloading to the original circumference is expressed as the value of a modulus:

$$M \text{ (circ)} = \frac{\text{load in Gm.} \times 980 \times \text{original circumference}}{\text{Cross sectional area in sq. cm.} \times \text{increase in circumference after unloading}}$$

Since stress is included in these formulas, the values obtained for the different groups can be compared at corresponding loads; they are shown in tables 1 and 2. A relatively low value of $M \text{ (ext)}$ at any load indicates a greater extensibility, or "tensility." A relatively low value of $M \text{ (retr)}$ at any load indicates a greater degree of retraction in terms of extended circumference. A relatively low value of $M \text{ (circ)}$ indicates a lesser ability to return to the original circumference after stretching.

RESULTS

Elastic Properties in Relation to Age.—Curves *A* and *B* in figure 3 show that the aortas of older rabbits (*A*) were more extensible than those of younger rabbits (*B*) under equal stresses. When, according to the method of Wilens,⁴ retraction was expressed as percentage of extended circumference (curves *a* and *b* in figure 3), practically no difference with respect to age was found. The aortas of older rabbits, although showing greater extension, did not retract to a correspondingly greater degree. This is further shown by curves *a'* and *b'* in figure 3, in which it is seen that the older aortas after stretching did not return as nearly to their original circumferences as did the younger aortas. In figure 4 the same ratios are plotted against logarithmic instead of numerical values of stress. The curves show the same relations to each other, but all are approximately straight lines, indicating that the elastic properties of the vessels are nearly true logarithmic functions of stress within the limits tested.

The data are presented in tabular form, based on five smaller age groups and expressed as values of the moduli described (table 1).

Here it is seen that all moduli increase with increase in load, indicating further that the elastic properties of extension and of retraction are not linear functions of stress. The values of *M* (ext) at corresponding

TABLE 1.—Average Values of Moduli for Elastic Properties of the Rabbit Aorta in Relation to Age

	Young			Old	
	6 Mo.	1 Yr.	2 Yr.	4 Yr.	5 Yr.
Number of rabbits.....	4	3	3	6	2
Number of segments of aorta tested.....	12	9	9	18	6
Average original circumference, mm.....	10.57	10.45	11.05	10.68	9.7
Average thickness of wall, mm.....	0.33	0.32	0.204	0.34	0.385
<i>M</i> (ext)					
at 20 Gm. of load.....	1.56×10^6	1.44×10^6	1.45×10^6	1.56×10^6	0.93×10^6
at 60 Gm. of load.....	2.07	1.78	1.98	1.72	1.40
at 100 Gm. of load.....	2.80	2.32	2.67	2.29	1.94
at 180 Gm. of load.....	4.31	3.55	4.14	3.50	3.02
<i>M</i> (retr)					
at 20 Gm. of load.....	2.23	2.40	2.32	2.12	1.72
at 60 Gm. of load.....	4.04	4.27	4.31	3.94	3.53
at 100 Gm. of load.....	6.05	5.93	6.47	5.87	5.36
at 180 Gm. of load.....	10.34	9.88	10.93	9.77	9.08
<i>M</i> (circ)					
at 20 Gm. of load.....	54.0	9.88	16.6	8.62	4.66
at 60 Gm. of load.....	42.8	16.1	26.7	14.2	8.26
at 100 Gm. of load.....	64.6	25.5	35.1	19.3	12.0
at 180 Gm. of load.....	90.8	44.5	57.1	31.8	19.4

TABLE 2.—Average Values of Moduli for Elastic Properties of the Rabbit Aorta in Relation to Level Tested

Location of segment of descending thoracic aorta.....	Upper	Middle	Lower
Number of segments of aorta tested.....	18	18	18
Average original circumference, mm.....	11.1	10.57	10.04
Average thickness of wall, mm.....	0.355	0.34	0.30
<i>M</i> (ext)			
at 20 Gm. of load.....	1.25×10^6	1.30×10^6	1.42×10^6
at 60 Gm. of load.....	1.67	1.63	2.00
at 100 Gm. of load.....	2.21	2.34	2.73
at 180 Gm. of load.....	3.36	3.58	4.23
<i>M</i> (retr)			
at 20 Gm. of load.....	2.04	2.12	2.38
at 60 Gm. of load.....	3.26	3.86	4.47
at 100 Gm. of load.....	5.48	5.77	6.70
at 180 Gm. of load.....	9.15	9.66	11.28
<i>M</i> (circ)			
at 20 Gm. of load.....	10.6	11.8	10.4
at 60 Gm. of load.....	14.6	18.4	18.2
at 100 Gm. of load.....	23.8	24.5	26.6
at 180 Gm. of load.....	37.6	39.7	38.3

loads become smaller with advance in age, showing a greater extensibility of the older aortas. For example, the average ratio of extension to original circumference at 180 Gm. of load was 124 per cent in the 6 month old group and 152 per cent in the 5 year old group. Although this relation is reversed in the case of the 1 and 2 year old groups, per-

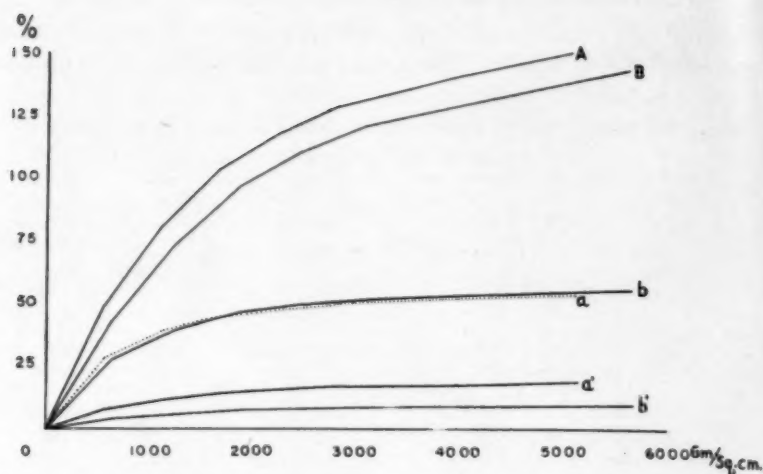


Fig. 3.—Graphic comparison of the elastic properties of young and old aortas. Curves *A* and *B* are made from the ratios of extension to original circumference of old and young aortas, respectively, plotted against numerical values of stress. Curves *a* and *b* show the ratios of retraction to extended circumference, and curves *a'* and *b'* show the ratios of increase in circumference after unloading to original circumference. The ratios are all expressed as percentages, and stress is given as the force in grams per square centimeter of cross sectional area.

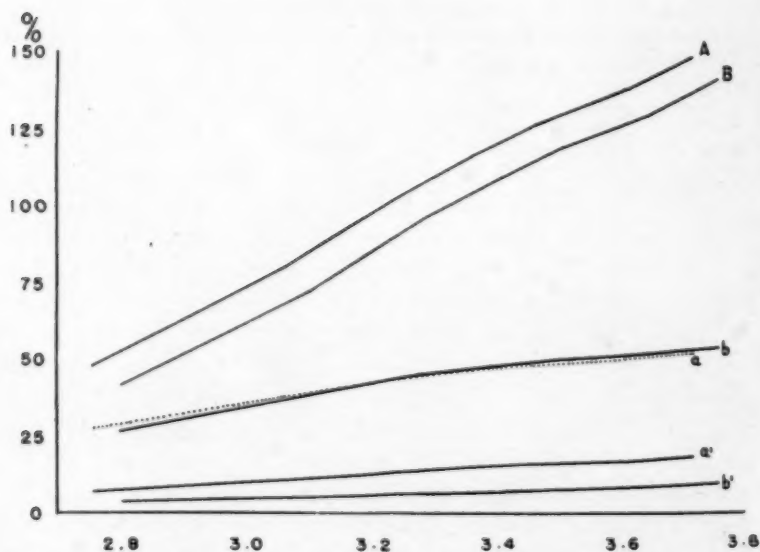


Fig. 4.—Values for the three ratios in figure 3 plotted against logarithmic values of stress.

haps because of the small number of animals, the values for these groups lie between those for the 6 month and 4 year groups. Differences in the values of M (retr) in relation to age are again slight and probably fall within the limits of error with this technic. The average ratios of retraction after 180 Gm. of load to extended circumference at this load varied among the five age groups from 50 to 55 per cent. The values of M (circ) show in general a progressive decrease with advance in age, indicating that aortas of old rabbits do not return so nearly to their original circumferences after stretching as do those of younger rabbits. After 180 Gm. of load had been removed, the average circumference in the 6 month old group was 6 per cent greater than the original circumference, whereas in the 5 year old group it was 24 per cent greater.

Elastic Properties in Relation to Location of Segment.—For this analysis the 54 segments tested were grouped irrespective of age but according to the three locations from which the segments were chosen; namely, the upper, the middle and the lower region of the descending thoracic aorta. The analysis was carried out in a manner similar to that described, and the data are shown in graphic form in figures 5 and 6. The values of the three moduli are given in table 2. These data show that the elastic properties of the rabbit aorta varied according to level. The amount of extension and of retraction at comparable stresses decreased progressively from the upper to the lower portion of the vessel as shown by curves 1, 2 and 3 and 1', 2' and 3', respectively, in figure 5. At all levels tested the aorta had on the average the same relative ability to approximate its original circumference after stretching (curves 1', 2' and 3' in figure 5). The values of the three moduli in table 2 show the same relations of elastic properties to the levels tested.

Morphology.—In tables 1 and 2 are given the average measurements of circumference and of thickness of wall of the aorta in the five age groups and at the three levels studied. The circumference was slightly smaller and the wall slightly thicker in old rabbits as compared with young ones. Since no tension was applied to the portions measured, it is possible that in their postmortem state the aortas of old rabbits are slightly contracted as compared with those of young rabbits. When the different levels are compared, there is a progressive decrease in circumference and in thickness of wall from the upper to the lower level. No difference was noted in the gross appearance of the aortas of young and old animals at corresponding levels.

Microscopic examination showed in all cases that the adventitia had been almost entirely removed by dissection. The few collagenous shreds of adventitia remaining would not seem to have exerted a measurable effect on the elastic properties of the segments tested. In no instance

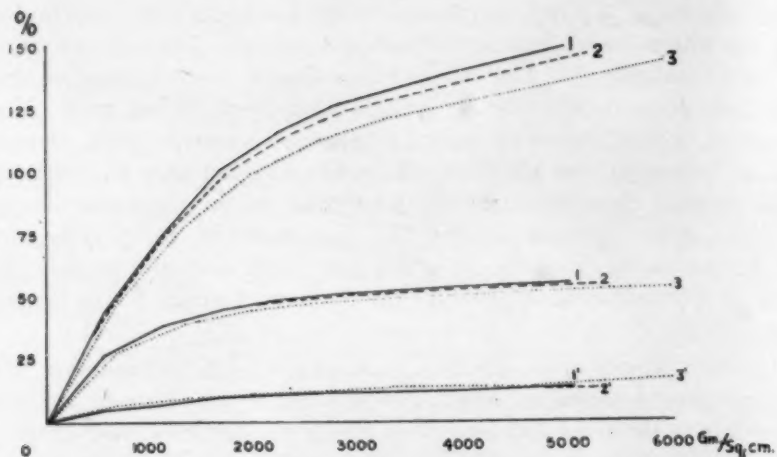


Fig. 5.—Graphic comparison of the elastic properties of three regions of the rabbit aorta. The three sets of curves are for the same ratios as in figure 3. In each set curves 1, 2 and 3 are made from values for the upper, middle and lower regions, respectively, of the descending thoracic aorta.

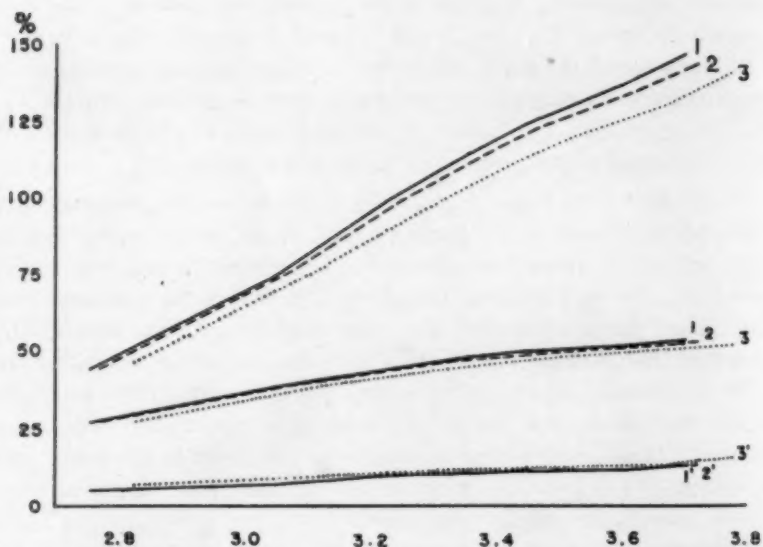


Fig. 6.—Values for the three ratios in figure 5 plotted against logarithmic values of stress.

was a thickened intima observed. It seemed probable that the elastic properties studied were chiefly those of the media and that differences in behavior of the segments could not be ascribed to differences either in the intima or in the adventitia.

A comparison of tested and untested portions of the same aortas showed no injury due to stretching. The one difference noted was that the elastic membranes were somewhat less wavy after stretching. This was most conspicuous in the aortas of older rabbits and was perhaps associated with their failure to return to the original circumference after extension.

Several changes with advance in age were noted microscopically with considerable regularity. The number of nuclei seen in the media was decreased in the older age groups. Abundant smooth muscle was seen in the media of young aortas. This appeared to be progressively replaced by collagenous tissue as age advanced. The elastic membranes appeared somewhat thinner and more widely separated in the aortas of the older rabbits, and the contours of the membranes were usually more wavy. Fine elastic fibrils were seen in the areas between the elastic membranes, and these were more numerous in the aortas of older rabbits. Microscopic differences dependent on location of segment were not seen.

COMMENT

The rabbit aorta becomes more extensible with advance in age. Hume^{7a} has shown that a similar change takes place in the rat aorta. In contrast, the human aorta with advance in age after maturity becomes less extensible.⁸ When retraction after nearly maximal extension of the aortas of rabbits is compared with the findings of Wilens⁴ for the human aorta, two differences are noted. The rabbit aorta does not show a corresponding decrease in retraction with advance in age, and the ratio of retraction to extended circumference at maximal load is greater for the rabbit aorta than for the human aorta at any age studied. Curves *a* and *b* in figure 3 show this ratio to be over 50 per cent, whereas the highest mean values given by Wilens for the human aorta are less than 40 per cent. The rabbit aorta was stretched to two and one-half times its original circumference as shown in curves *A* and *B* in figure 3, whereas the human aorta cannot be stretched to this extent. Furthermore, the rabbit aorta was not observed to reach a limit of maximal extension so sharply defined as that observed by Wilens⁴ and by Winternitz³ for the human aorta. This difference was seen to alter the shape of the loading curves in supplementary experiments in which segments of human aorta were tested in the manner described. In the case of comparable segments of aortas of infants, additional loads above 100 Gm. produced relatively little increase in extension, while segments of rabbit

aorta, although of about the same dimensions, continued to extend under these conditions. It is probable that the elastic properties of the aorta are characteristic of the species. A study of the rat aorta to be reported has shown that in this species the degree of extension at equal stress lies between that of the rabbit and that of man.

The changes in elastic properties of the rabbit aorta with advance in age are probably due to changes not in the elastic tissue itself but rather in its relation to other components of the vessel. Replacement of smooth muscle by collagenous tissue may so alter the properties of the vessel that in its intact postmortem state the vessel is slightly more contracted in old than in young animals. A slightly greater postmortem contraction would increase considerably the ratio of extension to original circumference; it would also help to explain why the older vessel is less able to return to its original circumference after being stretched. The method of Wilens⁴ for studying elastic properties does not require the original dimension for calculating amount of retraction. When the data for the rabbit aorta are analyzed by his method, no appreciable change in properties is found with advance in age. In Hume's experiments with the rat aorta⁷ retraction was not measured.

It is noteworthy that the rabbit and the rat do not show during their life spans the decrease in elastic properties of the aorta which has been observed in man. At an age when various involutional changes and major diseases are common in other organ systems, these animals do not show the amount of arterial disease found in man.⁹ This suggests that aging processes differ among the various species. In these rodents the aorta would seem to age less rapidly than some other organs, and the increase in extensibility of the aorta with advance in age to senility may perhaps be compared with the increase in extensibility of the human aorta during the first two decades of life.² The rate of aging of different organ systems would seem to depend on species characteristics. Loeb¹⁰ has shown that within a species (mice) there may be different rates of aging of the endocrine organs and the skeletal system dependent on strain characteristics.

The upper segments of aorta were consistently more extensible and retractable than the lower segments under equal conditions. Wilens⁴ found the opposite to be true for the human aorta at corresponding levels. No explanation was found for differences in elastic properties in relation to level or for the difference in this respect between the rabbit and the human aorta.

9. Cowdry, E. V.: *Arteriosclerosis*, New York, The Macmillan Company, 1933. Wilens, S. L., and Sproul, E. E.: *Am. J. Path.* **14**:201, 1938.

10. Loeb, L.: *Hormones and the Process of Aging*, in *Harvey Lectures*, 1940-1941, Baltimore, Williams & Wilkins Company, 1941, pp. 228-250.

In this study it has not been possible to determine how much of the elastic behavior of the vessel is due to each of its components or how much is due to the changes which take place in the elastic membranes under tension. When the microscopic picture is considered in relation to the great extent to which the rabbit aorta can be stretched, it seems probable that not only must the wavy membranes be straightened under tension but also these membranes must become stretched. The moduli for extension and retraction were found to increase with increase in load. Krafka⁶ has shown this to be true for the aortas of the cow, the dog and man. The values of Young's modulus for these species are slightly lower than those for the rabbit at comparable loads.

This method of studying elastic properties does not approximate physiologic conditions. Since tension is applied in one direction only, it is not possible to relate values of stress exactly to blood pressures, in which tension is applied in all directions. Values of stress cannot be converted directly into values of internal pressure. Formulas for conversions have been suggested by Dr. G. M. Hass and by Dr. LeR. L. Barnes, derived by different methods. These have indicated that a stress of about 1,000 Gm. per square centimeter of cross sectional area would correspond to a pressure of about 120 mm. of mercury. However, it is not clear that a linear relation exists between load and internal pressure. For purposes of this analysis it has not seemed necessary to draw too strict a comparison. It may be inferred that the steeper portions of each stress-strain curve, where values of M (ext) and M (retr) are lowest, probably correspond to the physiologic range of blood pressures.

SUMMARY

A study has been made of the elastic properties and morphologic character of the rabbit aorta at different ages (6 months to 5 years) and at three levels (upper, middle and lower regions of the descending thoracic portion). Segments (rings) of aorta, cut 5 mm. in length, from the three levels were subjected to increasing loads up to 180 Gm., and changes in their circumferences with and without loads were measured. From these measurements ratios of extension to original circumference, retraction to extended circumference and increase in circumference after unloading to original circumference were calculated. These ratios were compared graphically and as values of moduli at corresponding stresses in relation to age and to the level from which the segments were taken.

With advance in age the rabbit aorta becomes more extensible, shown by increasing values of the ratio of extension to original circumference and by decreasing values of Young's modulus at corresponding loads.

At the maximum load used the ratio of extension to original circumference was 124 per cent at 6 months and 152 per cent at 5 years.

No change in the capacity to retract after extension, expressed as the ratio of retraction to extended circumference, was observed in relation to age. At the maximum load used this ratio varied between 50 and 55 per cent.

With advance in age the rabbit aorta becomes less able to return to its original circumference after stretching, evidenced by increasing values of the ratio of increase in circumference after unloading the maximum load to original circumference (from 6 per cent at 6 months to 24 per cent at 5 years) and by decreasing values of the modulus for this relation at corresponding loads.

With advance in age the smooth muscle of the media is gradually replaced by collagenous tissue and there is an increase in fine elastic fibrils between the elastic membranes. The aortas of old rabbits, judged by measurements and by the waviness of the elastic membranes, appear slightly contracted in their postmortem state as compared with aortas of young rabbits, and these differences may be associated with differences in the elastic properties.

The upper portions of the aorta were capable of more extension and retraction than the lower portions under comparable conditions.

The observed changes in elastic properties suggest that the rabbit aorta, in contrast to the human aorta, does not age so rapidly as compared with other organs and is still a relatively young structure at the end of the life span.

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NUCLEOPROTEIN ANTIGEN OF VACCINE VIRUS

I. A NEW ANTIGEN FROM ELEMENTARY BODIES OF VACCINIA

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Infection with the virus of vaccinia gives rise in animals and in man to a number of antibodies which are specific for vaccinia-variola. At least four of these antibodies have already been discovered in hyperimmune serums; they are the neutralizing substances demonstrated by Sternberg¹ in 1892, the antibodies against the heat-labile (L) and heat-stable (S) soluble antigens described by Craigie and Wishart² and, finally, an agglutinin which has been designated X by Craigie and Wishart.³ L and X antisera are prepared from hyperimmune serum by absorption with S and LS antigens, respectively. S antiserum is generally obtained from rabbits after immunization of these animals with inactive elementary bodies of vaccinia. A recent article by Craigie⁴ provides a more detailed review of this subject. It is apparent that antisera prepared as described and designated L, S or X may contain additional vaccinal antibodies. The demonstration of another antibody in serums of animals after infection or hyperimmunization with the virus of vaccinia is the subject of this communication. In addition, a description of certain properties of the antigen responsible for eliciting the antibody is given.

MATERIALS AND METHODS

Elementary Bodies of Vaccinia.—Suspensions of elementary bodies were obtained by means of the differential centrifugation technic of Craigie⁵ from dermal pulp of rabbits which had been cutaneously infected with the lapinized C.L. strain of vaccine virus.^{6a} Pooled suspensions of elementary bodies were

From the Hospital of the Rockefeller Institute for Medical Research.

1. Sternberg, G. M.: *Tr. A. Am. Physicians* **7**:68, 1892.

2. Craigie, J., and Wishart, F. O.: *J. Exper. Med.* **64**:819, 1936.

3. Craigie, J., and Wishart, F. O.: *J. Bact.* **35**:25, 1938.

4. Craigie, J., in Doerr, R., and Hallauer, C.: *Handbuch der Virusforschung*, Berlin, Julius Springer, 1939, pt. 2, p. 1118.

5. Craigie, J.: *Brit. J. Exper. Path.* **13**:259, 1932.

5a. Smadel and others.⁶ Hoagland and others.⁷

concentrated by ultracentrifugation and after several additional washings were finally dried from the frozen state. Lots of dried virus with infective unit-elementary body ratios⁶ of 1:3 to 1:10 were employed. In certain experiments, materials discarded during the process of purifying the virus were also examined; these will be designated by the terms applied to them in an earlier paper.⁷

Procedure for Alkaline Extraction of Virus.—In general, the method of extracting virus was similar to that employed previously⁸; however, certain modifications have been made, and an illustrative example of a typical extractive procedure is given. In this instance 147 mg. of dried elementary bodies from a lot with an infective unit-elementary body ratio of 1:3.1 were washed in anhydrous ethyl ether and subsequently suspended in 45 cc. of distilled water. Rehydration was allowed to proceed overnight at 0 C.; then the suspension was warmed to 37 C., and 2.0 cc. of normal sodium hydroxide was added. The mixture was heated at 56 C. for fifteen minutes; it was then quickly chilled and partially neutralized with 2.0 cc. of half-normal hydrochloric acid. The material was immediately centrifuged in a chilled rotor at 30,000 revolutions per minute⁹ for one-half hour. The water-clear supernatant fluid was removed, brought to p_H 9.0 with the proper amount of half-normal hydrochloric acid and dialyzed overnight against five hundredth-molar lithium-barbital buffer solution, p_H 8.6. The 48 cc. of faintly opalescent extract of virus thus obtained was stored at 0 C. in barbital buffer solution until used. The total extract contained 68.5 mg. of material as estimated from the dry weight of a salt-free portion.

Antiserums.—Hyperimmune antivaccinal serums were obtained from rabbits immunized by repeated intravenous injections of saline suspensions of active elementary bodies. L antiserums were prepared from hyperimmune serum by absorption with heated dermal filtrate containing adequate amounts of S antigen. Both kinds of S antiserum used in the present work were obtained from non-immune rabbits kept under strict isolation in order to prevent accidental infection with the virus of vaccinia. To obtain the first kind, rabbits were given intraperitoneal or intravenous injections of a total dose of from 6 to 30 mg. of heat-inactivated elementary bodies over a period of weeks; to procure the second kind, Parker's method¹⁰ of immunization with purified S antigen¹¹ was employed with the exception that graded collodion particles¹² were added to the antigenic solution according to the suggestion of Salaman.¹³

Soluble Antigens of Vaccinia.—Dermal filtrate was prepared by extracting dermal pulp with one thousandth-molar disodium phosphate-citric acid buffer solution, p_H 7.2, and subsequently removing all virus by angle centrifugation and Seitz filtration.¹⁴ In this report, the terms "LS antigen" and "dermal filtrate" will be used interchangeably, and similarly "S antigen" and "heated dermal filtrate" will be considered as synonymous. The expression "purified S antigen" will be

6. Smadel, J. E.; Rivers, T. M., and Pickels, E. G.: J. Exper. Med. **70**: 379, 1939.

7. Hoagland, C. L.; Smadel, J. E., and Rivers, T. M.: J. Exper. Med. **71**: 737, 1940.

8. Smadel, J. E.; Lavin, G. I., and Dubos, R. J.: J. Exper. Med. **71**: 373, 1940.

9. Bauer, J. H., and Pickels, E. G.: J. Exper. Med. **64**: 503, 1936.

10. Parker, R. F.: J. Exper. Med. **67**: 361, 1938.

11. Parker, R. F., and Rivers, T. M.: J. Exper. Med. **65**: 243, 1937.

12. Shedlovsky, T., and Smadel, J. E.: J. Exper. Med. **72**: 511, 1940.

13. Salaman, M. H.: Brit. J. Exper. Path. **15**: 381, 1934.

used to designate material prepared by the technic of Parker and Rivers.¹¹ Degraded S antigen or S inhibitor¹⁴ will apply to the substance obtained by alkaline degradation of purified S antigen. The term "pure LS antigen" will be applied to the molecular substance of Shedlovsky and Smadel.¹⁵ Graded collodion particles coated with purified S antigen were prepared in the manner previously described.¹²

Serologic Methods.—All diluents used in serologic studies were buffered at pH 8.6 with hundredth-molar lithium-barbital. Buffered physiologic solution of sodium chloride was employed in the precipitin tests. In agglutination tests, suspensions of elementary bodies or of coated collodion particles were diluted to the proper degree of turbidity with hundredth-molar lithium-barbital buffer solution, and serial dilutions of antiserum were prepared in similar buffer solutions which, in addition, contained 0.45 per cent sodium chloride. In the precipitation and agglutination tests, 0.25 cc. volumes, respectively, of diluted antigen and of antisera were mixed, and the results were read after eighteen hours of incubation in closed tubes at 50 C. In the complement fixation tests 2 units of complement and overnight fixation at 0 C. were employed. Absorption experiments were carried out with optimal amounts of materials as determined by the methods of Dean and Webb.¹⁶ Inhibition tests were performed according to the technic employed previously.¹⁴

Chemical Methods.—Analytic methods were similar to those used in studies on the constituents of elementary bodies of vaccinia.¹⁷

EXPERIMENTAL OBSERVATIONS

For a number of years studies in this laboratory have been directed toward the isolation from vaccine virus of the substance or substances responsible for the production in animals of neutralizing antibodies and of resistance to infection. In recent papers from this⁸ and another laboratory¹⁸ methods have been described for extracting material from preparations of elementary bodies of vaccinia by means of dilute alkali. Such extracts were shown to be rich in nucleoprotein¹⁹ and to react serologically with antivaccinal serums.⁸ Furthermore, evidence was presented which was regarded as indicating that an appreciable amount of the serologically active material in the extracts was S antigen.⁸ It was pointed out, however, that S antigen as ordinarily obtained did not contain thymonucleic acid and that much of the material in the extracts which was flocculated by S antiserum appeared to be nucleoprotein. Whether this nucleoprotein constituted a new antigen or whether it

14. Smadel, J. E., and Rivers, T. M.: *J. Bact.* **39**:39, 1940.

15. Shedlovsky, T., and Smadel, J. E.: *Am. J. Path.* **17**:464, 1941.

16. Dean, H. R., and Webb, R. A.: *J. Path. & Bact.* **29**:473, 1926.

17. Hoagland, C. L.; Lavin, G. I.; Smadel, J. E., and Rivers, T. M.: *J. Exper. Med.* **72**:139, 1940. Hoagland, Smadel and Rivers.⁷

18. McFarlane, A. S.; MacFarlane, M. G.; Amies, C. R., and Eagles, G. H.: *Brit. J. Exper. Path.* **20**:485, 1939.

19. Smadel, Lavin and Dubos.⁸ McFarlane and others.¹⁸

represented a different and perhaps more nearly native form of S antigen could not be decided at that time. The data to be presented now indicate that the nucleoprotein is a previously unrecognized antigen of vaccinia.

Serologic Studies on Alkaline Extracts of Elementary Bodies of Vaccinia.—Typical results obtained in precipitin tests when serial dilutions of an alkaline extract of vaccine virus were treated with constant amounts of diluted L and S antisera are illustrated in table 1. It is apparent from the data presented that the extract contained a considerable amount of material that reacted with S antiserum. In addition, it contained a small amount of substance that precipitated in the presence of optimal amounts of L antiserum (a dilution of 1:6 in this

TABLE 1.—*Precipitin Reactions with an Alkaline Extract of Elementary Bodies of Vaccinia*

Antigen	Anti-serum	Dilution of Serum *	Precipitation with Given Dilution of Elementary Body Extract						
			1:4	1:8	1:16	1:32	1:64	1:128	1:256
Elementary body extract 54	S-274	1:4	++++	++++	++++	++++	++++	++	?
	L-6814	1:6	++	+	±	0	0	0	0
	L-6814	1:2	++++	++++	++++	+++	+	0	0
	Normal	1:2	0	0	0	0	0	0	0
Elementary body extract 54 heated at 56 C. for 1 hr.	S-274	1:4	++++	++++	++++	++++	++++	+++	+
	L-6814	1:2	++++	++++	++++	+++	±	0	0
	Normal	1:2	0	0	0	0	0	0	0
Dermal filtrate heated at 56 C. for 1 hr.	L-6814	1:2	0	0	0	0	0	0	0

* L-6814 serum in dilution of 1:2 was three times optimal. Other tests were done with antisera at optimal dilutions.

instance); on the other hand, the extract reacted to a higher titer when L antiserum was used in a more concentrated form (a 1:2 dilution). These reactions with L antiserum suggested that a material other than L antigen was responsible for the reaction. This was borne out by the fact that the precipitable substance was not inactivated by heating at 56 C. for one hour, whereas L antigen in dermal filtrate becomes non-precipitable after such treatment. The nature of the substance reacting with L antiserum was not immediately evident, but the precipitation with S antiserum, which had been prepared by immunizing rabbits with heated elementary bodies of vaccinia, led us to the conclusion that extracts of virus contain S antigen.

It was surprising, therefore, to find in subsequent work that no precipitation occurred when extracts of virus prepared in the usual way were tested against another type of S antiserum, namely, one obtained by immunizing normal rabbits with purified S antigen. The results summarized in table 2 show that two different extracts were precipitated

by antiserum 293 but not by antiserum 492, yet both antisera reacted with S antigen in heated dermal filtrate. The former serum had been obtained after immunization with heated virus; the latter, after immunization with purified S antigen.

It seemed likely that our original S antiserum, 293, contained precipitins for S antigen and precipitins for another antigen also present in extracts of virus. That such was the case is evident from the data

TABLE 2.—*Failure of Extract of Elementary Bodies to Precipitate with Antiserum Prepared Against Purified S Antigen*

Antigen	Antiserum	Dilution of Serum	Precipitation with Given Dilution of Antigen					
			1:4	1:8	1:16	1:32	1:64	1:128
Elementary body extract 76	S-293 (anti-heated elementary body)	1:6	++++	++++	++	+	0	0
	S-492 (anti-purified S)	1:2	0	0	0	0	0	0
Elementary body extract 80	S-293	1:6	++++	++++	++++	±	0	0
	S-492	1:2	0	0	0	0	0	0
Dermal filtrate heated	S-293	1:6	..	++++	++++	++	0	0
	S-492	1:2	..	00	++++	+++	+++	++
				+				

TABLE 3.—*Demonstration of Two Types of Antibodies in Antisera Prepared Against Heated Elementary Bodies*

Antigen	Antiserum			Precipitation with Given Dilution of Antigen						
	No.	Treatment	Dilution	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Dermal filtrate 16	293	Untreated	1:4	++++	++++	++++	+	±
		Absorbed with S antigen	1:4	0	0	0	0	0
Dermal filtrate 6	274	Untreated	1:4	..	++++	++++	++++	+++	+	?
		Inhibited with degraded S antigen	1:4	..	0	0	0	0	0	0
Elementary body extract 76	293	Untreated	1:4	++++	++++	+++	+	0
		Absorbed	1:4	++++	++++	+++	+	0
Elementary body extract 51	274	Untreated	1:4	..	++++	++++	++++	+++	+	?
		Inhibited	1:4	..	++++	++++	+++	+	?	0

shown in table 3. Antiserum 293 precipitated with dermal filtrate and also with extract of virus, but after absorption with S antigen it reacted only with the extract. Furthermore, antiserum 274, which had been obtained in a similar manner and which reacted well with both types of antigen, still precipitated the extract of virus after treatment with a sufficient amount of degraded S antigen to inhibit completely the S antibody present. Experiments of this type substantiated the idea that there is present in extracts of virus a substance that is serologically different from the heat-stable soluble antigen (S) of vaccinia.

Additional absorption studies were carried out to establish the identity of the precipitinogen in extracts of virus. It was found that antibodies against it could be removed from immune serum by absorption. Moreover, this could be done without greatly reducing the amounts of L and S antibody. The results obtained in a series of absorption experiments are summarized in table 4. These data show that solutions of LS and S antigens and of the antigen extracted from virus precipitated in dilutions of 1:64 to 1:128 in the presence of constant amounts of hyperimmune serum 1601. Absorption with S antigen and with LS antigen removed S antibody and L and S antibodies,

TABLE 4.—*Absorption of Hyperimmune Antivaccinal Serum with Several Antigens*

Anti-serum	Absorbed with	Test Antigen	Precipitation with Given Dilution of Test Antigen					
			1:4	1:8	1:16	1:32	1:64	1:128
1601 dilution 1:8	Untreated.....	LS.....	++++	++++	++++	+++	+++	++
		S.....	++++	++++	+++	++	+	0
		Elementary body extract	++++	++++	++++	+++	++	+
	S antigen.....	LS.....	++++	++++	++++	++++	+++	++
		S.....	0	0	0	0	0	0
		Elementary body extract	++++	++++	++++	+++	++	?
	LS antigen.....	LS.....	0	0	0	0	0	0
		S.....	0	0	0	0	0	0
		Elementary body extract	++++	++++	++++	+++	++	+
	Elementary body extract	LS.....	++++	++++	++++	++++	+++	++
		S.....	±	+	+++	++	0	0
		Elementary body extract	0	0	0	0	0	0
	LS antigen plus elementary body extract	LS.....	0	0	0	0	0	0
		S.....	0	0	0	0	0	0
		Elementary body extract	0	0	0	0	0	0

respectively, without reducing the capacity of the absorbed serum to react with an extract of virus. Absorption with extract of virus removed the precipitin for the antigen in the extract but left L and S antibodies. Finally, absorption with both LS antigen and extract of virus removed all demonstrable precipitins. This series of experiments clearly demonstrated that the precipitin which reacts with the principal serologically active constituent in extracts of virus is independent of L and S antibodies.

The next step was to establish the relationship of the newly recognized precipitin to the X agglutinin. The results of agglutination titrations carried out with unabsorbed hyperimmune serum 1601 and with portions of the absorbed serum used in the experiment discussed in the previous paragraph indicated that removal of all demonstrable

precipitins from hyperimmune serum leaves a residual agglutinin for elementary bodies. The data summarized in table 5 reveal that the agglutinin titer of the untreated serum was 1:2,048, that it was reduced by absorption with LS antigen and that it was also reduced by absorption with extract of virus. Moreover, after absorption with dermal filtrate followed by absorption with extract, the antiserum in a dilution of 1:256 still agglutinated elementary bodies. This remaining antibody was considered to be residual agglutinin.

TABLE 5.—*Agglutination of Elementary Bodies by Hyperimmune Serum*

Serum	Absorbed with	Agglutination in Given Dilution of Serum							
		1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
Normal	Untreated	±	0	0	0	0	0	0	0
1001	Untreated	++++	++++	++++	++++	++	++	+	±
	LS antigen	++++	++++	++++	++++	++	+	0	0
	Elementary body extract	++++	++++	++++	+++	++	+	0	0
	LS antigen plus elementary body extract	++++	++++	++++	+++	++	0	0	0

TABLE 6.—*Agglutination of Collodion Particles Coated with Purified S Antigen*

Antiserum	Treatment	Agglutination in Given Dilution of Serum						
		1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Hyperimmune 1001	Unabsorbed	++++	++++	++++	++++	+++	++	?
	Absorbed with LS antigen	0	0	0	0	0	0	0
Anti-purified S antigen 493	Unabsorbed	++++	++++	++++	+	0	0	0
	Absorbed with S antigen	0	0	0	0	0	0	0

An important question, namely, the effectiveness of the absorption technics, arises in attempting to evaluate the significance of residual agglutinin. Since each of the precipitins is capable of agglutinating elementary bodies under proper conditions, the reduction of these antibodies to a level at which they are no longer demonstrable by a precipitation technic might still leave sufficient amounts of them to be detected by the more sensitive agglutination technic. In several instances such a possibility was eliminated from consideration by demonstrating that the loss of precipitins for S antigen after treatment with appropriate amounts of dermal filtrate was accompanied by the disappearance of agglutinins for collodion particles coated with the purified S antigen (table 6). While these data are applicable only to S antibody, they

clearly indicate that when removal of this antibody is complete as indicated by precipitin tests, agglutinins for coated collodion particles are no longer demonstrable.

Craigie and Wishart⁸ defined the X antibody as a residual agglutinin which is demonstrable in antiserums after absorption with unheated dermal filtrate. Therefore, according to this concept, our new precipitin would be part of their X agglutinin. In view of the observations just reported it might be well to consider the X agglutinin of Craigie and Wishart as a group of several distinct antibodies, one of which reacts with the precipitinogen in our extracts of virus. Furthermore, for the time being, X agglutinin may be redefined as the residual agglutinin demonstrable in hyperimmune antivaccinal serum after removal of L and S antibodies and the antibody against the recently recognized precipitinogen in extracts of elementary bodies.

Attempts to associate the precipitinogen demonstrated in alkaline extracts of virus with the substance responsible for the production of neutralizing antibody and resistance to infection have been unsuccessful. These experiments are being continued and will be reported in detail at another time. It may be mentioned, however, that absorption of the new precipitin from hyperimmune serum does not appreciably affect the neutralizing power of that serum. Furthermore, immunization of normal rabbits with alkaline extracts of virus prepared in the usual manner readily elicits the production of antibodies for the precipitinogen in the extracts but not for LS antigen. Finally, rabbits immunized with extracts have no demonstrable resistance to infection with active virus.

The immunologic data presented indicate that the precipitinogen found in alkaline extracts of elementary bodies is unrelated to the previously recognized antigens of vaccinia. It is now also evident that the precipitable material which we demonstrated previously⁸ in extracts of virus and which we considered to consist largely of S antigen was in all probability entirely made up of this new antigen.²⁰ Since it has been shown that the precipitinogen is associated with the nucleoprotein fraction of the virus, it might be surmised that it is a nucleoprotein. Evidence for such a belief is presented in the following section; therefore,

20. Extracts prepared according to routine have not contained material that precipitates with S antisera from normal rabbits immunized with purified S antigen. This is accounted for by the fact that the method of extraction is probably sufficiently drastic to denature most of the S antigen present.⁸ In several instances in which less sodium hydroxide and heat were used in extracting the virus, small amounts of precipitable S antigen were detected in the preparations; for example, these extracts reacted in a dilution of 1:4 or 1:8 with S antibody (antiserum obtained by immunizing with purified S antigen) and had titers of 1:64 to 1:128 with the newly recognized precipitin (antiserum from rabbits given injections of extract of virus).

for simplicity the precipitinogen henceforth will be referred to as the nucleoprotein (NP) antigen of vaccinia.

Distribution of the Nucleoprotein Antigen and Its Antibody.—Dermal filtrate prepared in the usual manner is a rich source of LS antigen, but it has never yielded demonstrable amounts of the nucleoprotein (NP) antigen even when concentrated ten to twenty times. The NP antigen has been extracted by the usual method from materials discarded during the process of preparing purified virus from crude pulp. In each instance, however, the amount of antigen obtained on alkaline extraction was roughly proportional to the amount of virus present. Lots of dried washed elementary bodies were the best source of NP antigen; a greater yield of antigen per milligram of extracted dry material was obtained, the extracts precipitated in higher dilutions with NP antibody, and, finally, they contained less serologically inactive material of a non-nucleoprotein nature.²¹

Borate buffer solution, p_H 11.2, extracted practically all of the NP antigen from elementary bodies of vaccinia. This observation throws light on the results of the ultracentrifugation experiments of Beard, Finkelstein and Wyckoff,²² which they interpreted as indicating fragmentation of the virus of vaccinia into unsedimentable material and a heavier split product which sedimented at about one third the rate of elementary bodies.

The presence of NP antibody in serums of vaccine virus-immune rabbits which were hyperimmunized with active elementary bodies and in serums of normal rabbits given repeated injections of heat-inactivated elementary bodies is apparent from the data already presented. Moreover, it usually appeared in the serums of rabbits recovered from a simple infection with the virus. In normal rabbits which were immunized with heated virus the NP antibody appeared earlier and maintained a higher titer than did the S antibody; indeed, a number of samples of serum from such rabbits had appreciable amounts of NP antibody but no S antibody. In normal rabbits immunized with purified S antigen NP antibodies have consistently failed to develop. Pooled serums from guinea pigs which had been infected and subsequently hyperimmunized with washed elementary bodies contained L, S and NP antibodies in about equal amounts. In a cow infected with New York

21. Methods of extraction other than that described have been employed. Extraction with 1 per cent sodium carbonate of dried washed virus and of materials discarded during the process of purifying virus gave variable results, depending on the amount of material treated and the p_H of the resultant extract. In general, as one proceeded from the first discarded sediment to the final virus preparation the method yielded solutions containing decreasing amounts of S antigen and increasing amounts of NP antigen.

22. Beard, J. W.; Finkelstein, H., and Wyckoff, R. W. G.: J. Immunol. **35**:415, 1938.

City Board of Health calf lymph a small amount of NP antibody developed, which increased during hyperimmunization with active calf lymph. Thus, the NP antibody has been found in serum from several species of animals after infection with active virus or hyperimmunization with active or heat-inactivated elementary bodies of vaccinia.

Properties of the Nucleoprotein Antigen of Vaccinia.—The nucleoprotein (NP) antigen of vaccinia is practically insoluble in ordinary buffer solutions having p_H values between 4.5 and 7.0. It is precipitated in half-saturated solutions of ammonium sulfate and in 90 per cent ethyl alcohol. The antigen is soluble at p_H values above 8.0, and for this reason all serologic studies were carried out in solutions containing hundredth-molar lithium-barbital buffer, p_H 8.6. The NP antigen is relatively stable; it retains its serologic activity and immunizing properties for at least several months when stored in the cold; heating at 56, 70 and 95 C. for one-half hour causes no appreciable change in the appearance or in the precipitin or the complement fixation titers of solutions of the antigen, provided that the p_H is kept at around 8.6; the nucleoprotein can be repeatedly precipitated and redissolved by varying the p_H of the solutions without significant loss of serologic activity.

Chemical analyses on a number of alkaline extracts which had been dialyzed free of salts and dried from the frozen state gave approximately identical results, indicating the presence of 14.5 per cent nitrogen, 1.8 per cent phosphorus and 6 per cent nucleic acid of the thymus type. No significant differences were noted in analytic data obtained on extracts prepared according to routine and on nucleoprotein material which had been isolated from them by fractional precipitation at different p_H values or with ammonium sulfate. Approximately 1 part in 400,000 of the nucleoprotein (dry weight) was sufficient to give a precipitin reaction with optimal amounts of NP antibody.

Electrophoretic studies were made by Dr. Theodore Shedlovsky on several alkaline extracts of virus prepared according to routine and dialyzed against lithium-barbital buffer solutions, five hundredth-molar at p_H 7.9 and at p_H 8.7. From 90 to 95 per cent of the total material in the extracts was represented by an electrically homogeneous component which had a mobility of -6.0×10^{-5} cm./sec. per volt/cm. at p_H 7.9. In addition, there was a small amount of a second component that moved at -3.0×10^{-5} cm./sec. per volt/cm. Samples of the main component were isolated electrophoretically and were found to react with NP antibody. In extracts which had been heated at 70 C. for one-half hour there appeared a trace of an electrically inhomogeneous component which moved at an average rate of approximately -8.6×10^{-5} cm./sec. per volt/cm. This probably represented an altered portion of the main component, for the latter was slightly decreased in amount; however, the

mobilities of the main and minor components of the original material were essentially unaltered by the heat. The NP antigen has an electrophoretic mobility different from that of either pure LS antigen or heated pure LS antigen which was examined under similar experimental conditions.¹⁵ The slow component in extracts of virus moves at a rate close to that of the serologically inert fourth component in dermal filtrate.¹⁵

SUMMARY

A nucleoprotein which is found in alkaline extracts of elementary bodies of vaccinia constitutes a hitherto unrecognized antigen of vaccinia. This antigen is present in extracts of virus in amounts equivalent to 40 to 50 per cent of the weight of the elementary bodies. Like S antigen of vaccinia, with which it was confused in earlier experiments, the nucleoprotein (NP) antigen is heat stable. S antisera prepared by immunizing rabbits with heat-inactivated elementary bodies also contain NP antibodies in large amounts. NP antibodies have been demonstrated in serums of vaccine-immune and vaccine-hyperimmune animals of several species.

ENCEPHALOMALACIA WITH CAVITY FORMATION IN INFANTS

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In the past few years we have encountered brains of infants all presenting the same general picture to the naked eye and having practically the same histologic appearance. The characteristic feature of this condition is the presence of many well defined cavities in the white matter of the hemispheres, with a certain amount of hydrocephalus. Moreover, the condition can be diagnosed during the life of the infant by encephalography. The enlargement of the ventricles can be seen, and in many cases the cavities are filled with air, so that the picture is fairly characteristic of the disease (fig. 1 *B* and *C*). We have been particularly interested in the condition because 3 of the infants were syphilitic. The question has arisen, therefore, whether syphilis might be the cause of the encephalomalacia or whether at least some infectious process in the mother other than syphilis might be responsible for the condition. It seems quite unlikely that syphilis is the only cause of the condition, since there was no evidence of that disease in 4 of our 7 cases.

For the most part the cavities were confined to the white matter of the cerebral hemispheres. However, in some instances the cortex was not entirely spared. The cerebellum had no visible cavities in any of our cases, although in case 4 very definite histologic lesions were found where considerable numbers of Purkinje cells and the underlying granule cells had disappeared. In this case, also, the myelin was considerably thinned out in many places in the white matter of the cerebellar leaflets. Accumulations of fat were present in both the gray and the white matter, and the gliosis involved both the gray and the white matter. In only 3 instances were we able to make sections through the longitudinal sinus; in 2 of these it was patent, and in the third it was thrombosed. In only 1 instance was subdural hematoma encountered, and in only 1 was epidural hemorrhage present.

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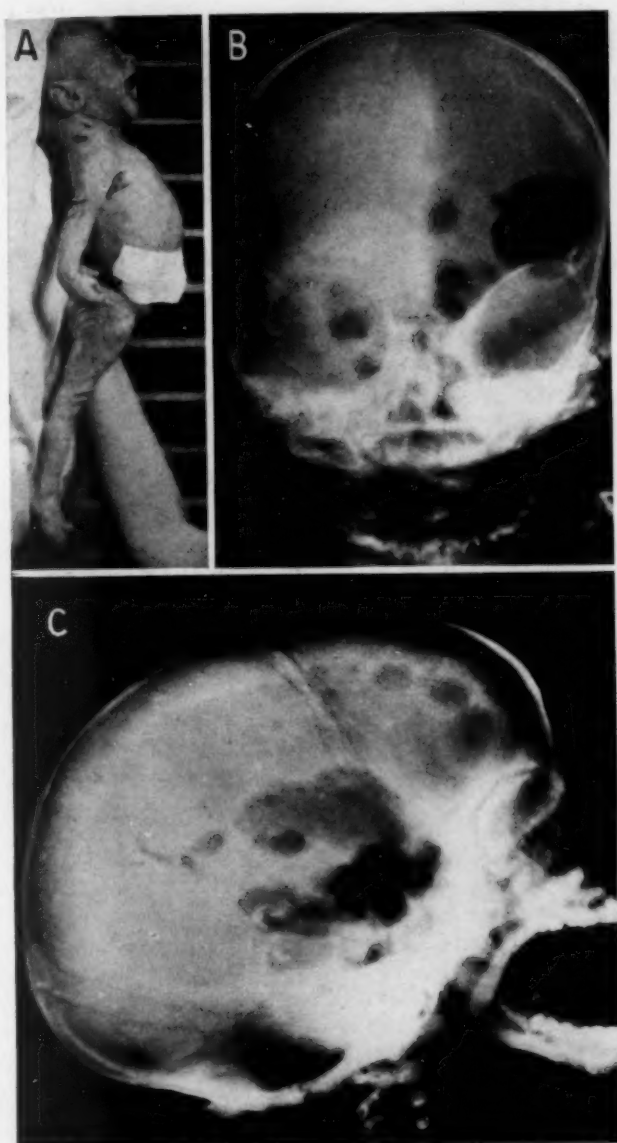


Fig. 1 (case 7).—*A*, convulsion produced by touching the infant. The infant was syphilitic and died at the age of 18 months. The picture was taken at the age of 4 months. *B* and *C*, encephalograms showing internal hydrocephalus and cavitation.

PREPARATION OF MATERIAL

Material was embedded in celloidin and sections stained with Masson's¹ trichrome stain, with hematoxylin and eosin and in some instances with Loyez^{1a} and Nissl stains. Material was also embedded in gelatin for frozen sections and sections stained by Hortega's methods for neuroglia and microglia; sudan III was combined with Hortega's nuclear stain in order to demonstrate the distribution of the fat.

CASE REPORTS

CASE 1.—A boy aged 3½ months was admitted to Bellevue Hospital Dec. 19, 1937. His mother was one of fourteen children, seven of whom died of marasmus in infancy. She had three healthy children, aged 8, 7 and 5 years. The mother had frequent colds and tonsillitis prior to the birth of this child, and at four months after conception had a severe attack of vomiting lasting about twelve hours, which caused subconjunctival hemorrhages. The Wassermann test of her blood was negative. Labor occurred at 8½ months, resulting in a normal spontaneous delivery. The infant at birth showed slight asphyxia, due to mucus and slight external molding of the head, but no external defects. The weight at birth was 5 pounds 9 ounces (2,523 Gm.). From birth he continued to have attacks of cyanosis. The jaw was held rigid, and he was unable to nurse. The forearms were held flexed and occasionally were tremulous. On the fifth day the spinal fluid was under pressure and appeared almost like whole blood, but after 5 cc. was removed it became clear. The fontanelles were bulging. After three weeks the baby continued having cyanotic attacks at night. He became rigid when touched. He was taken home at this time, and since he had never been able to nurse, he was fed with a medicine dropper. He gained only slightly in weight.

On admission to Bellevue Hospital he was dehydrated. The skin was dry and loose. The skull sutures were prominent and were beginning to override. The head circumference was 13½ inches (34 cm.). The baby did not respond to visual or auditory stimuli. He was in opisthotonos and was undergoing intermittent slow convulsions with generalized spasticity and sometimes rapid jerking movements of the arms and legs. Any external stimulus, such as handling, would start a convulsion. The pupils were equal and regular and reacted sluggishly to light. The external ocular movements were normal. There was purulent rhinitis. The pharynx could not be seen because of the spasticity of the jaw. The knee and ankle jerks were active. The course of the illness was complicated by nasopharyngitis, fever, and terminal bronchopneumonia at the age of 4½ months.

The Wassermann test of the blood was negative. The blood revealed 5,050,000 red cells and 18,000 white cells. The blood culture was negative. Lumbar puncture revealed 40 lymphocytes per cubic millimeter. The Wassermann test of the spinal fluid was negative. Roentgen examination of the skull showed a maximum anterior-posterior diameter of 12 cm., 4 cm. less than is normal for this age. There were no other changes of the skull. Dentition was normal. Encephalography with injection of 75 cc. of air showed that the lateral ventricles were dilated, the left more than the right. The third and the fourth ventricle were also enlarged. There was a large quantity of air subtentorially in the cisterna magna, outlining

1. Masson, P.: *J. Tech. Methods* **12**:75, 1929.

1a. Wertham, F.: *The Brain as an Organ*, New York, The Macmillan Company, 1934, p. 69.

the cerebellum. The left cerebral hemisphere was surrounded by an amount of air in the subdural space. There were small focal accumulations of air in the frontal, temporal and occipital lobes.

Postmortem examination revealed emaciation and hypostatic lobular pneumonia. Histologically there was acute fibrinous pleuritis and bronchlobular pneumonia. The spleen showed acute splenitis and hyperplasia of the reticulum and pulp cell series. The liver and the kidneys were normal.

At removal of the brain it was noted that there was a distance of about $\frac{1}{2}$ inch (1.27 cm.) between the calvarium and the cortex. The brain showed partial aplasia of both frontal lobes with quite marked widespread cortical atrophy. In contrast the cerebellum appeared almost normal in size. There was a small sub-

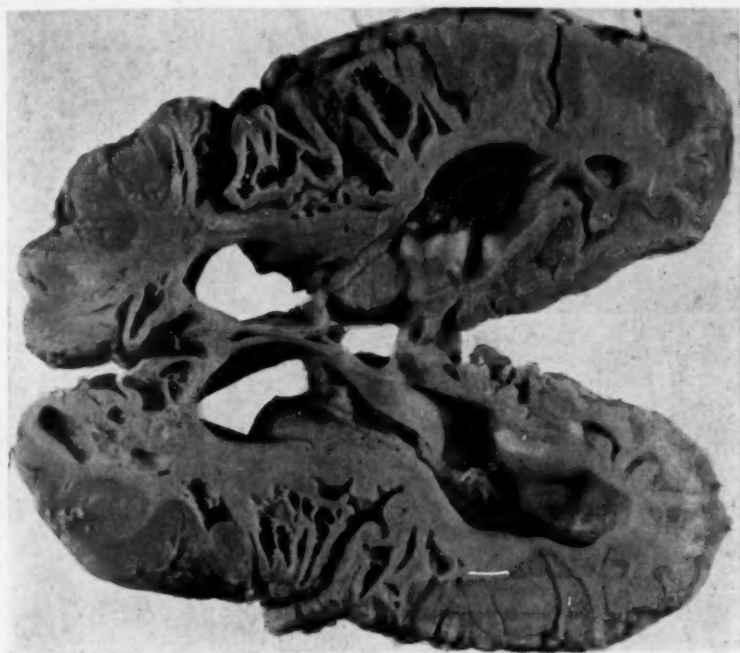


Fig. 2 (case 1).—Horizontal section through the brain of an infant aged 4½ months, showing diffuse trabeculated cavity formation.

arachnoid hemorrhage in the right occipital pole. There seemed to be some atrophy of the posterior part of the cerebral hemispheres as well as of the cerebellum projecting beyond these. The brain cut with some difficulty. There was cystic degeneration of the subcortical white matter in both temporal lobes covering the insula and in both frontal poles. There were pinhead-sized holes throughout the basal ganglia. There was slight internal hydrocephalus, apparently due to atrophy of the brain. The brain stem and the cerebellum showed no gross lesions.

Histologically, the large trabeculated cavities in the subcortex were lined with gitter cells. There were many rarefied areas containing gitter cells where the tissue was not completely lost. In some parts the cortex was not normal and contained many rarefied areas, in which were large cells with peripherally placed

nuclei. The cortex was most severely involved where the overlying meninges were thickened. There were large amounts of fat throughout the sections, in the cortex, subcortex, basal ganglia, pyramidal tracts of the midbrain and medulla.

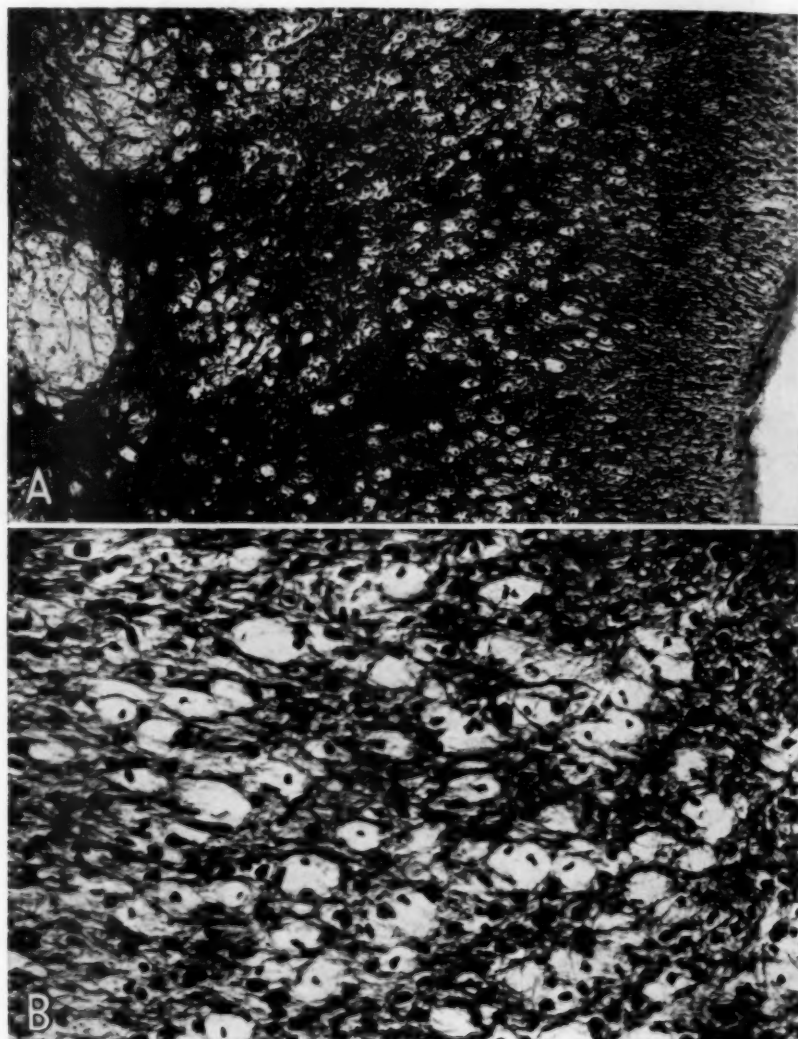


Fig. 3 (case 1).—*A*, low power magnification of diffuse gliosis of the cortex and subcortex, with many fat-containing cells, in the brain of an infant aged 4½ months. Hortega's neuroglia stain. *B*, high power magnification of diffuse gliosis of the cortex and many large cells filled with fat in the same brain. Hortega's neuroglia stain.

There was an interlacing of neuroglia fibers in the cortex and surrounding the cavities. The occipital lobe showed similar lesions but was not as intensely involved. The blood vessels were normal.

In the basal ganglia were many small cavities, similar to those described in the cortex. These cavities were unrelated to blood vessels. There were also large collections of gitter cells, fat and neuroglia.

The midbrain did not show the cavity formation, but there was an increase in neuroglia in the periaqueductal region and small amounts of fat in the cerebral peduncles.

In the medulla there was free fat in the pyramidal tracts. The cranial nerve nuclei were normal. There were no areas of softening in the cerebellum. The neuroglia was increased in the white matter. The Purkinje cells were normal.

CASE 2.—A boy, the second of twins, was delivered by breech extraction at Bellevue Hospital Aug. 12, 1937. The mother's pregnancy was normal and uncomplicated by toxemia. The Wassermann test of her blood was negative. She had secondary anemia at term: red blood cells 3,660,000 and hemoglobin content 70 per cent. Both infants appeared normal at birth and were transferred to the premature babies' ward. The first twin died within three weeks, of intestinal intoxication, but the brain was not examined. The second twin had a low grade fever during the first two weeks, after which the temperature became normal. He became moderately jaundiced and listless and took feedings poorly. He had occasional attacks of cyanosis. There were no convulsions or spasticity. He gradually lost weight and died in a marasmic state at the age of 1 month. A Wassermann test of the blood was not made.

Autopsy showed marasmus, prematurity and patchy atelectasis of the lungs. The pancreas showed pericapsular and interstitial hemorrhage. The liver and the spleen were congested and in the lungs were areas of alveolar collapse.

The brain appeared normal on removal. There was no hemorrhage or tear of any of the membranes. The sinuses contained no thrombi. In the right hemisphere, in the posterior part of the frontal lobe just lateral to the wall of the lateral ventricle was a trabeculated cystic cavity, measuring 1.5 by 1 cm. It did not communicate with the lateral ventricle and extended outward to the subcortex. In the left hemisphere, in the corresponding region there was a similar area of softening just perceptible grossly, with early tiny cavity formation.

Sections from the centrum ovale and cortex disclosed large and small cavities, lined irregularly with gitter cells, and in many parts were free red blood cells. There were a few hemorrhages throughout the sections and marked congestion of blood vessels. The meninges were slightly thickened. The blood vessels appeared normal. There were numerous areas of rarefaction with loss of cells throughout the cortex. No definite ependymal lining of the lateral ventricle was seen; irregular ependymal canals extended below the wall of the ventricle.

There was an increase in fat throughout the areas of softening together with large focal accumulations of fat globules. The neuroglia was markedly increased, not only around the cavities but also about the blood vessels and in the subependymal region.

There was marked gliosis in both the gray and the white matter; this was particularly noticeable about the blood vessels; there was not, however, the typical distribution of fat-containing cells in the interstices as in cases 1 and 5.

Section through the longitudinal sinus demonstrated it to be normally patent.

CASE 3.—A Negro girl 6 months old was admitted to Bellevue Hospital, Sept. 18, 1936, because she had been having "spasms all day long" since being placed with a foster mother, ten days before. She was the child of a 19 year old unmarried mother and was born on March 2, 1936. Nothing is known of the mother's pregnancy or labor or of the infant before placement with the foster mother except that she presented no feeding difficulty.

On admission she showed good nutrition. Any slight stimulus would produce a convulsion. Loud noises provoked a seizure. In a typical convulsion the entire body stiffened and assumed a modified opisthotonic position. The right arm was held in a hand-shaking position; the left, in the same position but higher and more abducted. The head turned to the right and the eyes to the left. The head was brachycephalic, and the anterior fontanel was closed. The eyes did not follow a light or a moving object, and the infant did not blink when an object was suddenly passed before her eyes. The optic disks were pale. The neck was flaccid, and the head could not be held alone. The deep reflexes were hyperactive, and a Babinski sign was present bilaterally. The Kernig sign was positive, and there was a positive Brudzinski sign. Ankle clonus was present on the left. The skin on the arms and legs showed a papular eruption; the papules were often confluent in large patches. The epitrochlear nodes were enlarged. The lungs were clear, and examination of the heart disclosed a murmur in the second left intercostal space.

The Wassermann test of the blood was negative. The serum calcium amounted to 10.8 mg. and the serum phosphorus to 5.7 mg. per hundred cubic centimeters. Cisternal puncture disclosed a clear cerebrospinal fluid with 10 cells, 75 per cent of which were polymorphonuclear leukocytes. The Wassermann test of the spinal fluid was negative. Roentgen study of the skull showed it to be of the microcephalic type. The anterior fontanel was almost completely closed. Roentgenograms of the long bones disclosed no abnormality.

The frequency of seizures was decreased by sedatives. The infant's condition ran an afebrile course for two months. She was fed by gavage during this period. November 18, her temperature rose to 105 F., and she was found to have lobar pneumonia. *Pneumococcus* type XIV was cultured from the blood, pleural fluid and cerebrospinal fluid. She died November 23, at the age of 8 months and 3 weeks, five days after the onset of pneumonia.

Postmortem examination revealed pneumococcic pneumonia, acute pneumococcic leptomeningitis, acute suppurative otitis media on the left, generalized hyperplasia of the lymph nodes and hyperplasia of the thymus. Histologic examination showed bronchopneumonia.

On removing the calvarium, the dura stripped with slight difficulty, and its inner surface appeared deeply hemorrhagic, with many fibrinous shreds visible along the longitudinal sinus. The subarachnoid space contained an increased amount of fluid, which was of a milky color. After fixation the brain presented a congenital anomaly with marked diminution in the size of both frontal lobes and definite microgyria. The left temporal lobe was quite defective in development. The cerebellum was unusually large in proportion to the rest of the brain. Coronal section showed some dilatation of the lateral ventricles and small cavities in the white matter, particularly in the left hemisphere.

Histologic examination revealed practically no myelin on coronal section of the hemisphere. There were many cavities in the white matter filled with phagocytes. Large amounts of fat were demonstrated in phagocytes and free in the white matter. Neuroglia fibers were increased in the gray matter.

There was a large amount of fat in both the gray and the white matter of the brain. The distribution of the neuroglia fibers and the fat-containing cells in the cortex and subcortex was very similar to that seen in cases 1 and 5.

CASE 4.—A boy 5 months of age was admitted to Bellevue Hospital Jan. 22, 1939. He was born Aug. 21, 1938, at term, but the labor lasted three days. In the second month of pregnancy the mother had taken pills which induced one day

of bleeding. She was under ether anesthesia during the last six hours of parturition and had uterine hemorrhages before the baby was born. The obstetrician had difficulty in delivering the head and shoulders. The infant weighed 7 pounds 14 ounces (3,572 Gm.) at birth, and cried and breathed normally.

At 17 days he had his first convulsion. At 4 weeks he was admitted to the hospital because of fever (105 F.), cyanosis and unconsciousness. The blood calcium value was found to be 7 mg. per hundred cubic centimeters, and a diagnosis of tetany was made. He was removed to another hospital Oct. 3, 1938, at 2 months of age, where he was found to be spastic and to have positive Chvostek and Trousseau signs. The serum calcium amounted to 13 mg. per hundred cubic centimeters, but the spinal fluid calcium was found to amount to 0.7 mg. per hundred cubic centimeters. The baby was treated with calcium chloride, drisdol (a preparation of crystalline vitamin D₂), viosterol and ultraviolet rays, but the spasticity persisted with a spontaneous scissors position of the legs, and marked abductor spasm developed. Bilateral optic atrophy was noted. During the three months that the baby was in the hospital, nasopharyngitis, bronchitis and otitis media developed. He was transferred to Bellevue Hospital when 5 months old.

On admission he showed good nutrition and had slight generalized lymphadenopathy. He had a hemangioma of the groin. The sutures of the head were overlapping; the pupils did not react to light, and there was bilateral optic atrophy. The reflexes could not be accurately determined because of the general spasticity. He had continuous athetoid movements involving the trunk, neck and shoulder muscles. Under sedation and during sleep the spasticity decreased. The infant lived in the hospital for one year and died at the age of 17 months. During this period he had febrile episodes with bilateral otitis media. The spasticity increased, and he maintained a nearly constant opisthotonic position. Convulsions continued throughout with only slight stimulation. He died in a marasmic state.

The laboratory findings were within normal limits except that leukocytosis accompanied the febrile episodes. The blood and spinal fluid gave negative Wassermann reactions. The spinal fluid contained 10 mononuclear cells. Encephalography at 12 months demonstrated marked dilatation of the right and the left ventricular system and what appeared to be communicating cavities on one side.

Postmortem examination revealed cervical and mediastinal lymphadenitis, bilateral pleural effusion and pulmonary atelectasis. Histologic examination showed visceral congestion.

The calvarium was removed with difficulty because of the adherence of the dura along the suture lines. The dura appeared hemorrhagic and gelatinous in consistency and was 3 to 4 mm. in thickness. After fixation of the brain it was noted that the dura, including part of the longitudinal sinus, showed a fibrous thickening with a small lumen. On both sides of the sinus was a well rounded chronic subdural hematoma. The cisterna magna was filled with blood. Blood was present also in the subarachnoid space. The brain showed marked microgyria. The left temporal lobe was ballooned out in three sections and had a thin translucent cortex. Part of the superior frontal gyrus on the left was similarly ballooned out, but to a less extent. The anterior part of the right temporal lobe was also much thinned out. The cerebellum appeared normal. The brain cut with great difficulty. On further section of the brain, there were revealed multiple cavity formations, most marked in the temporal lobes and less extensive in the frontal lobes.

Histologically, the longitudinal sinus had a small lumen surrounded by a large amount of fibrous tissue. In the superior frontal gyrus were many subcortical foci

of necrosis. There was practically no myelin in this section. In many of the necrotic areas were large numbers of small round bodies that stained like calcium. There was a considerable amount of fibrous tissue formation in some of the necrotic areas. The neuroglia was greatly increased in the neighborhood of the small cavities. Large deposits of fat were found in and about the necrotic foci; it was mostly contained in phagocytes. The cerebellum was fairly normal in appearance, although in places there was a loss of granular and Purkinje cells and many of the remaining Purkinje cells were atrophic. In the acellular areas calcium had been deposited. There was very considerable loss of myelin in places in the cerebellar leaflets.

CASE 5.—A girl was admitted to Bellevue Hospital at the age of 1 month. The weight at birth was 4 pounds (1,814.5 Gm.). The mother when six months pregnant had a 4 plus Wassermann reaction of the blood and was given two intravenous injections. About twenty-four hours before the baby was brought to the hospital, gastroenteritis developed, with blood in the stools. The infant was pale, premature and dehydrated. There was an exfoliation on the sole of the left foot. The liver and the spleen were enlarged. The temperature was 107 F. She became quite icteric. Antisyphilitic therapy was given in the form of bismo-cymol. There were no convulsions or neurologic signs. Lobar pneumonia developed, and the child died at the age of 9 weeks.

The red blood count was 1,800,000; the white blood count, 16,000. The Wassermann reaction of the blood was not recorded. Roentgenograms of the long bones showed the characteristics of congenital syphilis.

Postmortem examination revealed emaciation, icterus, lobar pneumonia, syphilitic cirrhosis of the liver, splenomegaly and syphilitic periostitis. There was a peribulbar and pericellular syphilitic cirrhosis of the liver; also, confluent fibrinopurulent pneumonia. The bones showed syphilitic osteochondritis and periostitis with fibrosis of the marrow. The spleen showed passive congestion. The kidneys, pancreas, heart and adrenals were normal.

The calvarium appeared normal. The dura stripped with ease. The sinuses were normal. After fixation of the brain in formaldehyde solution-bromide solution, it was noted that there was a marked difference in the size of the cerebral hemispheres. The right was much smaller than the left, and there was microgyria of the right occipital pole. There was slight hydrocephalus of the right lateral ventricle. Cystic degeneration was found in the subcortical white matter in the right hemisphere superior to the lateral ventricle, involving somewhat the gray substance of the cortex and extending from the frontal to the occipital poles. The left hemisphere, brain stem and cerebellum were not affected.

Histologically, in the right parieto-occipital lobe were large trabeculated cavities lined with gitter cells. In parts, the meninges were thickened, consisting of a thick outer layer of fibroblasts and a loose underlying structure containing gitter cells and mononuclear cells. There were numerous areas of rarefaction in the underlying cortex, which showed a marked increase in neuroglia fibers and focal accumulations of small round nuclei. There was hyaline thickening of the adventitia of the medium-sized arteries, but there was no evidence of syphilitic endarteritis or of inflammatory response. Fat in the subcortical region was markedly increased. Myelin could not be demonstrated in the sections examined. Neuroglia was increased in the periaqueductal region, but otherwise the left hemisphere, midbrain and medulla showed no lesions.

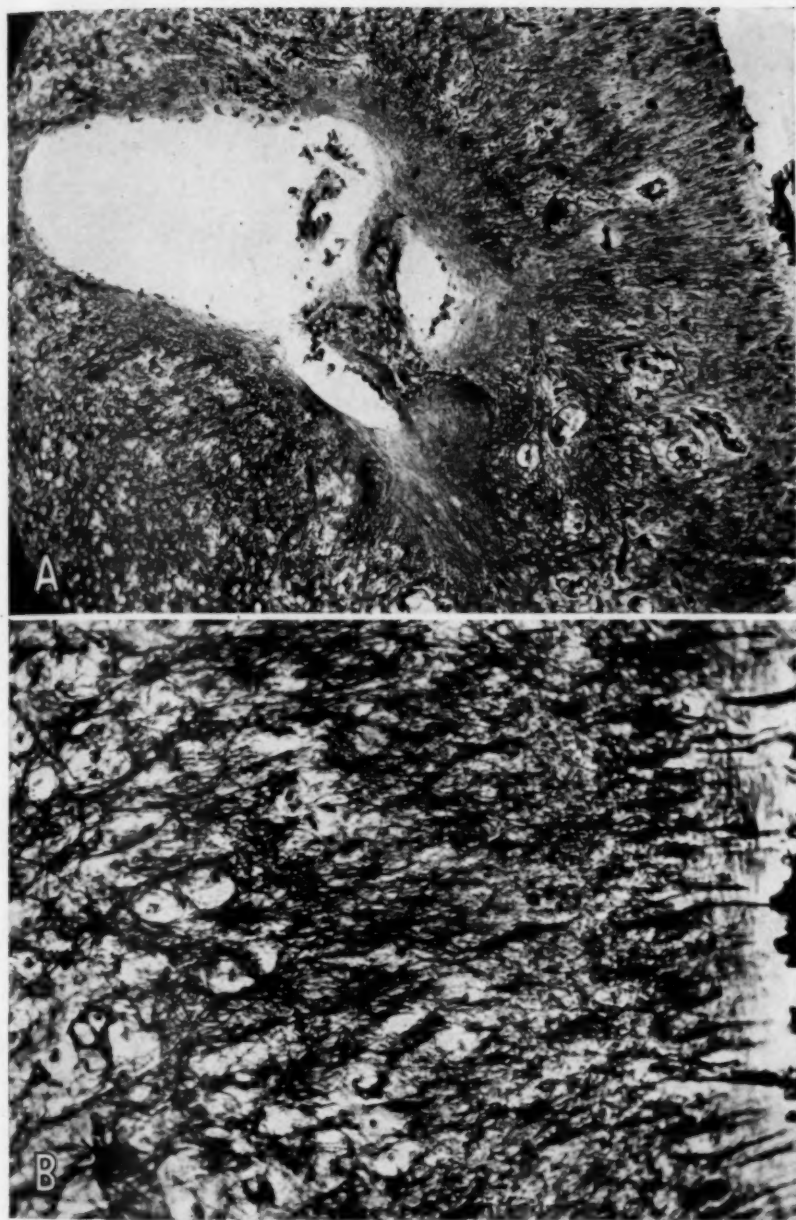


Fig. 4 (case 5).—*A*, low power magnification of cavity formation about the juncture of the cortex and the subcortical white matter, showing marked formation of glia fibers about the cavities and in the brain, with large fat-containing cells, particularly in the white matter. The patient was 9 weeks old and syphilitic. Hortega's neuroglia stain with sudan III. *B*, high power view of the cortex, showing marked gliosis in the gray matter, with large fat-containing cells in the gray matter. Hortega's silver carbonate stain for neuroglia with sudan III.

CASE 6.—A boy was admitted to Bellevue Hospital at the age of 14 days. The mother had a positive Wassermann reaction of the blood, discovered at the time of delivery. The period of gestation was probably six and a half months. The delivery was spontaneous, after a labor of three hours. On admission the infant was emaciated and weighed 2 pounds, 5 ounces (1,049 Gm.). There was over-riding of the bones of the skull. He had gonorrheal ophthalmia. His mouth was infected with thrush. There were attacks of cyanosis, but no convulsions or neurologic signs. The temperature was subnormal. The Wassermann reaction of the blood was 4 plus. The infant had frequent watery stools, became listless and died at the age of 1 month.

The gross anatomic diagnoses were prematurity, marasmus and patchy atelectasis of the lungs. Histologic examination revealed patchy alveolar collapse of the lungs. The kidneys, liver, spleen and bones were normal.

On removal of the brain there was no tear in any of the membranes, nor any evidence of hemorrhage. The dural sinuses were free of thrombi. After fixation in formaldehyde solution-bromide solution, the brain showed no atrophy. There was no evidence of basilar meningitis. In the centrum ovale of each hemisphere were somewhat hemorrhagic necrotic areas.

Sections through the area of softening showed the brain to be broken down into a series of small cavities. These cavities were lined with gitter cells and occasional red blood cells, and there were definite small hemorrhages throughout the softening area. In a section through part of the ventricle wall there was a small raised nodule covered with ependyma that appeared to be a gumma. For the most part, it contained lymphocytes and a considerable number of gitter cells and other mononuclear cells. There was some hemorrhage within the central portion of this mass, with dilatation of the capillaries near the mass. Some of the vessels in this section had mononuclear cellular infiltration of the adventitia. There were large amounts of fat in the region about the cavities and extending outward to the cortex. Astrocytes were definitely increased in the region of the cavity formation.

CASE 7.—A girl was born at term in Bellevue Hospital, Jan. 16, 1938, without complications. She weighed 6 pounds, 10 ounces (3,000 Gm.) at birth and appeared normal. The Wassermann reaction of the blood was positive at birth. The mother was known to be syphilitic, but she had not received antisyphilitic therapy during this pregnancy, and came to the hospital only after labor had begun. The infant appeared normal during the first ten days; then snuffles developed and there were attacks of cyanosis, during which the arms quivered. A temperature of 102 F. and the cutaneous lesions of congenital syphilis developed. Lumbar puncture at this time gave negative results, and the Wassermann reaction of the spinal fluid was negative. At 1 month generalized spasticity developed. The child lay in marked opisthotonos, and there were frequent extensor movements of all four extremities, provoked by the slightest stimulus. The deep reflexes were hyperactive, and there were contralateral abductor responses on eliciting the knee jerks. There was coarse nystagmus. The fundi showed well outlined disks and were somewhat pale, with many pigmented spots of pinpoint size, especially in the left fundus.

During the second, third and fourth months the baby was given an intensive course of intravenous injections of neoarsphenamine and intramuscular injections of a bismuth compound, and the Wassermann reaction of the blood became negative. An air encephalogram was done at 2½ months; there was a small amount of air

in the right lateral ventricle, the left ventricle was dilated, and there were encysted areas of air in the left temporal region. A diagnosis of encephalomalacia with cavity formation was made from the encephalogram (fig. 1 *B* and *C*). The course in the hospital over a period of eighteen and one-half months, when death intervened, Aug. 2, 1939, was gradually downhill and punctuated by many intercurrent infections—diarrhea, bronchopneumonia and otitis media with several bouts of fever. After the first six months the patient remained rigid, lying in a markedly opisthotonic position with the head rigidly fixed to the right and the legs in extensor rigidity and fixed in scissors position. There was bilateral optic atrophy. The anterior fontanel was sunken and the head small in size. The opisthotonic position gradually reached a stage at which the head was nearly touching the buttocks (fig. 1 *A*).

On March 8, 1938 the Wassermann reaction of the blood was 4 plus; on June 2, negative. On Feb. 2, 1938 the spinal fluid cell count was 14, with 11 lymphocytes and 3 monocytes; the Wassermann reaction was negative; on March 18, the cell count was 42 lymphocytes, the Pandy reaction 4 plus, the Wassermann reaction negative and the colloidal gold curve 0000000000; August 2, the cell count was 30 lymphocytes and the colloidal gold curve 3333321000. An air encephalogram June 16 revealed that the lateral ventricles were tremendously dilated, extending almost to the frontal bones. There were small clusters of air collections in the sub-arachnoid spaces anteriorly and posteriorly.

Postmortem examination revealed advanced emaciation and visceral congestion.

The calvarium was removed with difficulty. The dura was adherent throughout its extent. There was an epidural hemorrhage on the tentorium cerebelli close to the margin. The straight sinus and the transverse, superior petrosal and cavernous sinuses were thrombosed throughout. As the dura was opened, about 100 cc. of clear yellow fluid escaped. After fixation with formaldehyde solution-bromide solution, the cerebral hemispheres were much shrunken in size, and the dura covering them was thickened and adherent to the pia-arachnoid, which was also thickened. The temporal lobe was cystic, with a thin wall. The cerebellum, the pons and the medulla appeared to be grossly normal. Section through the hemispheres showed marked encephalomalacia with small cavities in places.

Histologically, the dura was considerably thickened and attached in places to the leptomeninges and the cortex. There were many hemorrhagic areas and dilated blood vessels within the dura. The cortex of the brain immediately underneath showed some preservation of the gray matter. There were many small empty spaces in this rim of cortical gray substance and many phagocytes and lymphocytes occupying the space where the white matter should be. There was an increase also in the amount of fibrous tissue in the space where the white matter had disappeared. The leptomeninges contained large numbers of phagocytes and some increase of the fibrous tissue. The blood vessels seen in this section were congested but otherwise normal. Sections stained for neuroglia revealed an abundance of reticulum fibers but very few preserved neuroglia cells or fibers. There was a considerable amount of fat in phagocytes in the section through the optic thalamus, distributed about the blood vessels and in the substance of the thalamus. The neuroglia fibers were greatly increased in this section. The appearance in the thalamus was similar to that seen in the cortex in cases 1 and 5, with large cells in the interstices.

In the longitudinal sinus was a fairly well organized thrombus, occupying the entire lumen.

Virchow,² in 1883, described congenital encephalitis and myelitis with which he found circumscribed and diffuse collections of gitter cells and fat droplets in the white matter, rarely in the gray matter. These he regarded as fatty metamorphosis of the neuroglia. He considered the condition to be an irritative process caused by inflammation.

Globus,³ in a contribution to the histopathology of porencephalus, apparently describing a condition different from that dealt with here, stated that the majority of cortical blood vessels were cut off by meningeal cicatrization and others were strangulated by glial changes and were often obliterated and converted into narrow bands of connective tissue. Furthermore, he found inflammatory lesions in the midbrain, the pons and the medulla. He stated that the changes in the brain were clearly inflammatory in origin.

Sternberg⁴ described a case in which he found in the white matter of the brain of a boy 16 months of age, smooth-walled cavities lined by glial bundles, with smaller cystic cavities filled with fat-laden cells. Apparently, however, he had no explanation to offer as to the etiologic factors. He mentioned cases reported by Cruveilhier in 1832, in which the lesions were thought to be due to trauma, and also a case described by Schwartz in which it was thought that the etiologic factor might be trauma.

Innes⁵ described a disease found in lambs as "swayback," and that disease is in some ways similar to the condition we have described. The animals showed atrophy of the brain, some hydrocephalus, widespread degeneration of the white matter with cavity formation, relative sparing of the gray matter and marked gliosis, but apparently no increase of lipoid. There was loss of myelin, however. The disease of lambs might have been due to some toxic substance in the pasture where these animals lived.

Diamond⁶ described 2 cases of encephalomalacia in infants. In the first case, cavities were found in the brain with proliferation of glia, which was thought to be of prenatal origin and of toxic or infectious nature. However, there was partial detachment of the retina, and the case may have belonged in a different group. In the second case there were changes in the other organs, for example, the liver, the heart and the kidneys, and the condition was thought to be of toxic origin.

2. Virchow, R.: *Arch. f. path. Anat.* **38**:129, 1867; **44**:472, 1867; *Berl. klin. Wchnschr.* **46**:705, 1883.

3. Globus, J. H.: *Arch. Neurol. & Psychiat.* **6**:652, 1921.

4. Sternberg, C.: *Beitr. z. path. Anat. u. z. allg. Path.* **84**:521, 1930.

5. Innes, J. R. M., and Shearer, G. D.: *J. Comp. Path. & Therap.* **53**:1, 1940.

6. Diamond, I. B.: *Arch. Neurol. & Psychiat.* **31**:1153, 1934; **32**:676, 1934.

Ford,⁷ who described 3 cases of this condition, called it "disseminated encephalomalacia with cavity formation," and in discussing the etiologic factors he concluded that it seemed probable that the condition was due either to a toxic or to a degenerative process.

Bailey and Hass⁸ expressed the belief that certain acquired cerebral defects in infants are due to thrombosis of the superior longitudinal sinus. They expressed the opinion that this condition explains some instances of focal cerebral gliosis, "encephalitis" in childhood and spontaneous subdural or subarachnoid hemorrhages.

In considering this subject, it has been necessary to keep in mind the work of Wolf, Cowen and Paige,⁹ who have described a new form of encephalitis in children, which they term "toxoplasmic encephalomyelitis." In the brains of these infants there is necrosis, but also there is abundant evidence of an inflammatory process in the brain and an associated focal chorioretinitis which can be observed during the life of the infant.

SUMMARY

In this paper we have described 7 cases of encephalomalacia with cavity formation in infants because the condition has not received much attention in the medical literature and because of the great similarity of the cases to one another, as if in all of them the disease had been the result of the same etiologic factor. Unfortunately, our examination of these cases does not indicate what the etiologic factor is. The cases in which congenital syphilis was also present suggest that the disease could be produced in utero by toxemia resulting from an infectious process of some kind. The condition does not seem to depend on thrombosis of venous sinuses in the dura, since in the 2 cases of our series in which the longitudinal sinus was sectioned it was found to be patent. The disease apparently does not depend on any arterial abnormality of the brain, for there was no notable change in the arteries in any of the sections examined. Only in case 6, and in this instance the patient was syphilitic, was there any evidence of encephalitis. In this case we found small granulomatous nodules under the ependyma of the lateral ventricles, as well as periarteritis of some of the vessels deeper within the brain substance.

In only 3 cases of our series was anemia of some degree present. It was severe in only 1 case. One infant had a high fever at the age of 1

7. Ford, F. R.: *Diseases of the Nervous System in Infancy, Childhood and Adolescence*, Springfield, Ill., Charles C. Thomas, Publisher, 1937, p. 271.

8. Bailey, O. T., and Hass, G. M.: *Brain* **60**:293, 1937.

9. Wolf, A.; Cowen, D., and Paige, B. H.: *Am. J. Path.* **15**:657, 1939.

month. Two infants had lymphadenopathy. Apparently, the condition was present at birth or made itself manifest by neurologic signs shortly thereafter.

The histologic appearance of some if not of all of these brains suggested that the condition had been present long before birth. The mothers were all young women, so that the age of the mother apparently had no bearing on the condition in the child. Some of the mothers had other children, who were normal. It does not seem that the duration or the complications of labor had any bearing on the condition. There were no particular complications of pregnancy in most of the cases.

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DEVELOPMENTAL ABNORMALITIES OF THE LUNG AND BRONCHIOGENIC CARCINOMA

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The association of bronchiectatic and cystic cavities with primary cancer of the lung is an old observation. Whenever present, such cavities have generally been considered the result of bronchial obstruction and secondary infection, caused by the tumor's occlusion of the lumen of the bronchus. That such dilatations may sometimes be the result of developmental defects rather than of the presence of the cancer is suggested to us by the frequency with which tissue disorganization can be demonstrated in the walls of such dilatations.

Cellular differentiation is the end result of normal cellular development. Where this cellular development is incomplete or abnormal, faulty differentiation may result with concomitant tissue disorganization and morphogenetic malformation. The frequent association of cancer in such sites may not be entirely fortuitous. It is a well known fact that areas of tissue malformation withstand those environmental stresses and strains that stimulate tissue growth much more poorly than do normal, well differentiated cells. This can be observed in other portions of the body when such congenital lesions as the black mole or neurofibroma are injured and, as a result, seem to acquire a more rapid rate of growth and invasive characteristics.

In two recent communications we have called attention to the association of abnormal pulmonary development and tissue overgrowth. In one of these¹ we described a type of tumor usually beginning in the bronchial wall and protruding into, and often occluding, the bronchial lumen. While usually composed of epithelium resembling fetal lung tissue, these tumors occasionally contain mesodermal elements, and for that reason we chose to call them "mixed tumors." Many of the tumors diagnosed as "bronchial adenoma" are tumors of this type. These tumors give a fairly characteristic clinical and pathologic picture and therefore can usually be differentiated preoperatively. The question of extension is not so easily determined, however. They do invade locally; we have

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1. Womack, N. A., and Graham, E. A.: *Arch. Path.* 6:165, 1938.

evidence that they may later give rise to distant blood stream metastasis, at which time local growth is more rapid and there are associated changes in cellular appearance. The developmental abnormalities most often associated with such tumors seem to be related to lobe and bronchus formation. There have been seen accessory minor bronchi, accessory lobes or incompletely formed lobes. Occasionally, cyst formation has been encountered.

For our second publication² we examined carefully lungs and lobes removed at operation the condition of which was diagnosed as congenital cystic disease. The criteria for the establishment of this diagnosis were the absence of many of the normal structures usually found in the bronchial wall as far down the bronchial tree as the respiratory bronchiole. If there was no evidence that normal organization had ever been present, we felt that abnormal development must be assumed. It must be borne in mind that the development of lung continues for a number of years after birth,³ and therefore factors having to do with developmental failures are not necessarily limited entirely to the intrauterine life. Where such aberrations were found, we were able to demonstrate that epithelial overgrowth in the form both of hyperplasia and of metaplasia occurred. These areas of growth were usually multiple and often microscopic. They could be differentiated from clinical cancer only in extent and in rate of growth. In our opinion this offered additional evidence that certainly in many instances the origin of a tumor of the lung was concerned with abnormal cellular differentiation.

In the present communication we have chosen to select several types of developmental abnormalities that we have found associated with bronchiogenic carcinoma as seen in lungs removed at operation. These represent only a few of the different types of lesions that we have encountered in this association. These will all be considered in greater detail, as well as the literature related to the subject, in a subsequent and more lengthy report.

REPORT OF CASES

CASE 1.—In a white married woman 24 years old pneumonia developed in January 1941, two months after a difficult delivery. The pneumonia was atypical, and one month later a slightly productive cough developed. The patient noticed loss of energy, shortness of breath on exertion and on two occasions sharp pains of the lower left side of the chest, aggravated by breathing. The sputum was never bloody or rusty. There was a loss of 16 pounds (7 Kg.) in weight in the following year. She had consulted her physician early about her complaint and had received three bronchoscopic examinations.

Her past history and the family history failed to reveal any information of significance with regard to the present complaint.

She was admitted to Barnes Hospital Feb. 18, 1942. Diminished fremitus was noticed in the left side of the chest posteriorly, below the fourth rib. There were

2. Womack, N. A., and Graham, E. A.: *Am. J. Path.* **17**:645, 1941.

3. Willson, H. C.: *Am. J. Anat.* **41**:97, 1928.

diminished breath sounds in the same area. No rales were heard. The left side of the diaphragm descended poorly. The temperature was 37.7 C. (99.8 F.), the pulse rate 75 and the respiratory rate 18. The vital capacity was 1,700 cc. The urinalysis showed a faint trace of albumin. No tubercle bacilli were found in various examinations of the sputum. The blood gave a red cell count of 4,040,000 with a hemoglobin content of 10.4 Gm., and a white cell count of 13,350, with a normal differential count. The nonprotein nitrogen of the blood amounted to 12 mg. per hundred cubic centimeters; sugar, to 85 mg. The Kahn reaction of the blood was negative. The electrocardiogram was normal.

A bronchographic examination was made February 19. There was good filling of the trachea and of only the proximal part of the left main bronchus. This bronchus was markedly narrowed, and none of the opaque material extended farther than 2 cm. from the point of origin of the bronchus. A diagnosis of occlusion of the left main bronchus was made.

A bronchoscopic examination was made February 20 by Dr. Brian Blades. The left main bronchus was found to be almost completely occluded by a mass of tumor tissue at a point 3 cm. distal to the carina. When tissue was taken for biopsy, slight bleeding was encountered. Microscopic examination of this tissue showed the bronchial epithelium to be squamous in type. Beneath this was an area of loose fibrous tissue being invaded by masses and groups of clear, vacuolated epithelial cells, tending to form alveoli and resembling somewhat the cells seen in the hypernephroma type of renal carcinoma. Because of the invasive nature of these cells it was felt that this tumor should be considered clinically cancerous.

March 2 total left pneumonectomy was performed (E. A. G.) with separate ligation of the vessels and the bronchus. Except for the development of a small leak in the stump of the bronchus which required drainage of the pleural cavity, the postoperative course was uneventful.

Gross Appearance of Specimen.—The specimen removed at operation consisted of the entire left lung (fig. 1). The pleural surface was smooth and shiny everywhere, and there was a pinkish yellow color except over the upper lobe, which was considerably darker and more cyanotic. There were many strong fibrous adhesions between the two lobes; otherwise the fissure was well demarcated. At the hilus of the upper lobe there could be seen protruding from the main bronchus a large soft friable mass, which completely filled the bronchus. The diameter of the bronchus appeared greater than that usually encountered. There were a few enlarged lymph nodes at the hilus of the lung, which on section showed no evidence of cancer. The lung was sectioned. The lower lobe presented a moderate degree of bronchiectasis without, however, any evidence of infection. The bronchi were dilated and tortuous, but the walls appeared only slightly thickened. In the parenchyma of the lower lobe there were numerous small cystic areas with thin walls and no gross evidence of surrounding pneumonitis. Similar cysts were seen in the outer portion of the upper lobe, some of which were filled with what was apparently mucus and tumor tissue. These varied in size from 2 mm. to 1 cm. A cyst in the center of the upper lobe was completely filled with tumor tissue. The upper lobe presented a moderate amount of atelectasis but nowhere was there any evidence of pus. At the hilus of the upper lobe there was a large, round mass completely filling the main bronchus, apparently perforating the bronchial wall and extending also down the bronchus. It was about 3 cm. in diameter.

Microscopic Appearance of Involved Lung.—An entire sagittal section of the lung was cut into separate blocks and studied microscopically. The cell type can be seen in figure 2 A, B and C. The sections shown were taken through the large tumor at the hilus and included the bronchial wall and the adjacent lymph nodes. The cells



Fig. 1.—Two views of the lung in case 1, showing different sagittal planes. In *A* the main tumor can be seen extending out into the surrounding lung. In *B* a slightly enlarged photograph shows several abnormal bronchi and numerous cysts, some containing tumor tissue.

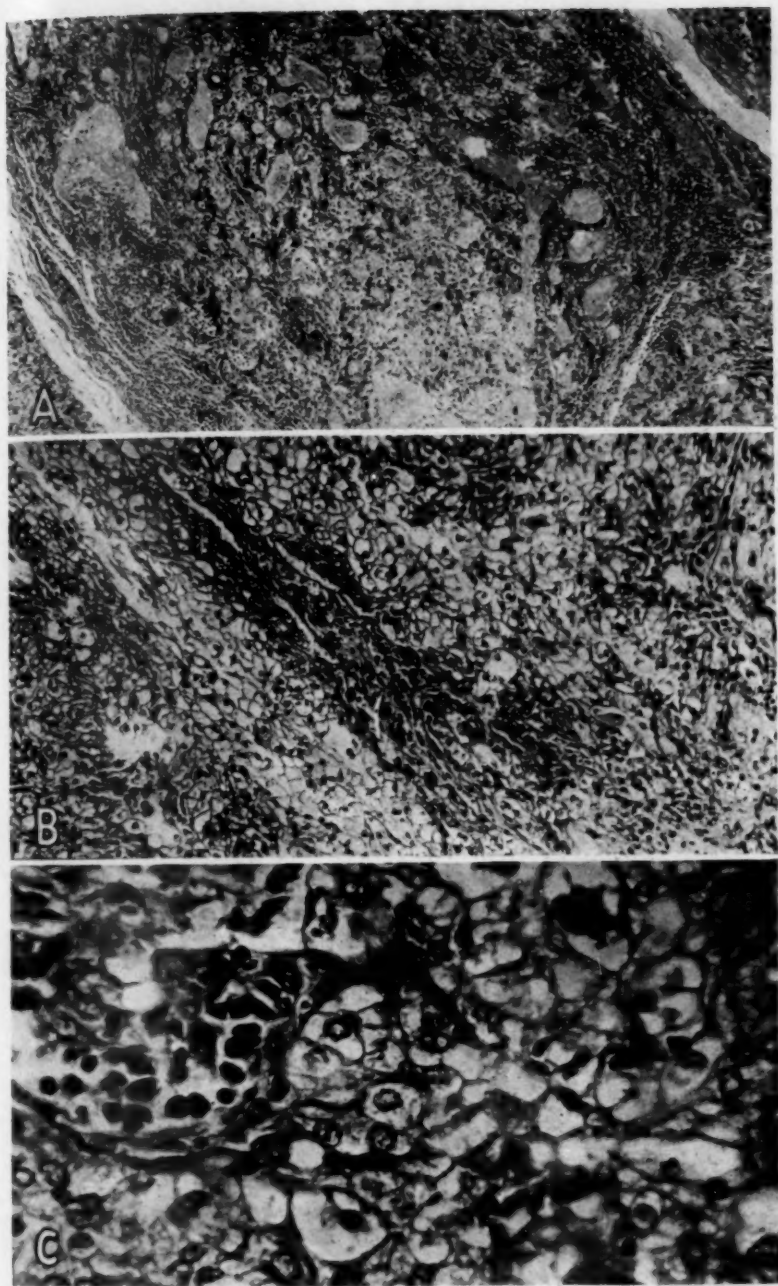


Fig. 2.—All three photographs illustrate a tumor of a main bronchus in case 1: *A*, low power view showing complete invasion of the bronchial wall. There is a tendency to form numerous small cystic dilatations, which are filled with mucus. *B*, marked vacuolation of the tumor cells giving the appearance of hypernephroma. *C*, high power view of tumor cells showing considerable variation in nuclear appearance.

are large and vacuolated, with nuclei of irregular size and shape. Some of the vacuoles appear to be filled with mucus, and the cells show a tendency to form alveoli, the cavities of which are filled with mucus. Mitotic figures are present

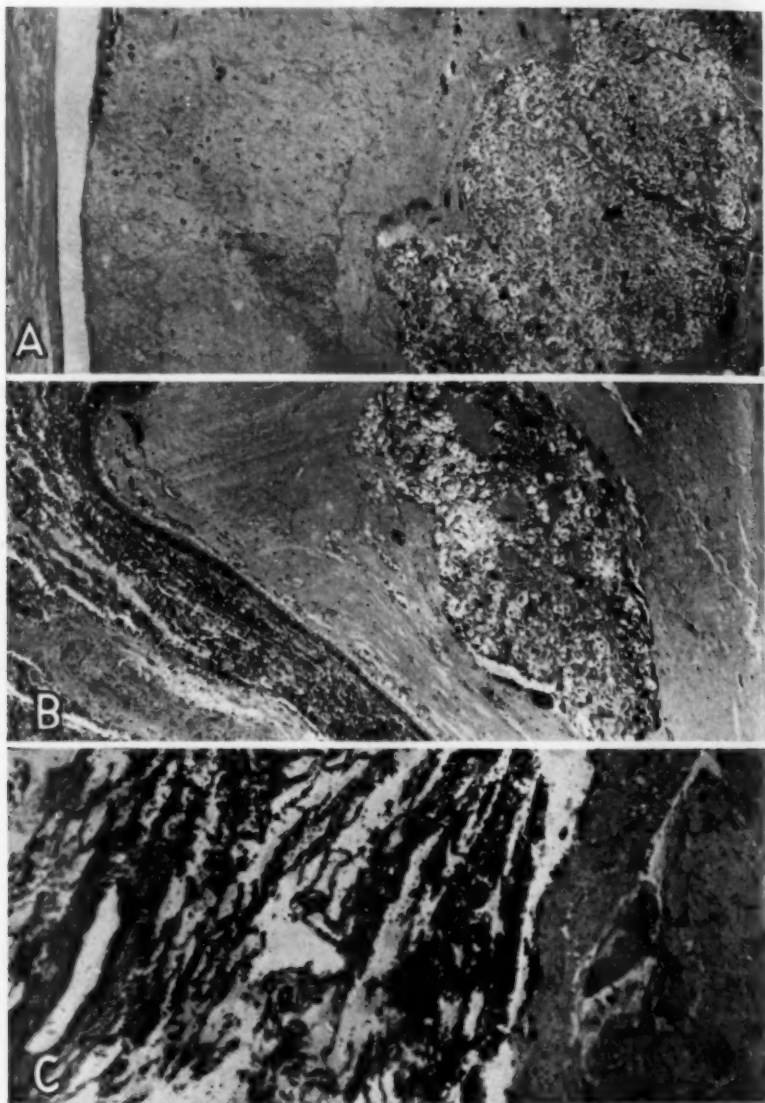


Fig. 3 (case 1).—*A*, cystic cavity lined by low cuboidal epithelium containing mucus, in which is growing tumor tissue in the form of cystadenoma. *B*, smaller cyst showing a picture similar to that described in *A*. *C*, thin-walled cyst in the periphery of the lung, showing little cellular activity.

but not common, and one is impressed by the apparent slow growth of the lesion. In figure 2 *A* it can be seen that the entire bronchial wall has been permeated and

destroyed. The tumor tissue has invaded the peribronchial tissue and is adjacent to a lymph node. Figure 3A and B shows separate cystlike structures containing tumor tissue. These areas are well away from the large tumor and probably represent independent sites of tumor formation. The same type of tumor cell is present, growing in mucus and in 3A apparently extending as a papillary projection from the cells lining the cyst. Other cysts showed no evidence of tumor formation but presented simple epithelium-lined cavities in which few of the structures normally encountered in a bronchial wall were present. There was little evidence of atelectasis of the surrounding pulmonary parenchyma such as one would expect to be present if these were respiratory bronchioles that had undergone recent and marked expansions (fig. 3C).

CASE 2.—A 54 year old white man was admitted to Barnes Hospital Sept. 19, 1940. His health had been good until six months prior to admission, when a non-productive cough, weakness and fever developed. His temperature was elevated from 2 to 3 degrees each afternoon. His illness was considered influenza by his family physician, and rest in bed was prescribed. He remained in bed four months, at first with little improvement; later his strength returned and he began to regain weight so that he felt able to work again. During this time he had lost 27 pounds (12 Kg.). The cough persisted, as did the fatigue. The symptoms of fever and malaise reappeared in one month, and the cough became productive. He returned to bed but began to have pain over the left side of the lower part of the chest. This was constant and was aggravated by coughing. There were several episodes of hemoptysis.

His past history and family history were not relevant to the present complaint.

There was dullness over the upper lobe of the left lung anteriorly in addition to dullness and diminished breath and voice sounds over the lower lobe. Fluoroscopy revealed atelectasis of the lower lobe. The left side of the diaphragm was fixed.

The red blood cell count was 4,200,000; the white cell count, 9,600; the hemoglobin content was 80 per cent. The hemogram was normal. All other laboratory findings were within normal limits except the electrocardiogram, which showed evidence of myocardial damage. The vital capacity was 3,400 cc.

A bronchogram showed no iodized oil entering the bronchus to the lower lobe of the left lung, which appeared to be completely occluded at its origin from the left main bronchus. The lingular division of the upper lobe of the left lung was not seen. Several of the bronchi of this lobe showed cylindric bronchiectasis.

Bronchoscopic examination by Dr. Thomas Burford revealed a tumor projecting into the lumen of the left main bronchus 2.5 cm. from the carina. A portion removed for microscopic study showed interspersed through areas of fibrous tissue masses of cancerous squamous epithelial cells growing without direction or pattern. Mitotic figures were occasional.

October 10 total left pneumonectomy was done by Dr. Brian Blades, with separate ligation of the bronchus and vessels. Involvement of the lymph nodes in the region of the superior pulmonary vein was thought to be present at the time of operation, although this could not be verified.

Convalescence was delayed by the development of empyema, which was drained. Evidence of metastasis to the pubic bone appeared, which was associated with considerable pain. This pain was relieved by roentgen therapy. The patient was discharged from the hospital November 20 and readmitted March 22, 1941 with evidence of metastases in the liver. He subsequently died while away from the hospital, and permission for an autopsy was not obtained.

Gross Appearance of Specimen.—The material consisted of the left lung (fig. 4), the fissure between the lobes of which was not complete. Both lobes were sectioned.

The structural pattern of the lower lobe was markedly distorted due to extensive diffuse bronchial dilatation and cyst formation. The dilatation extended almost to the pleural surface, and the involved bronchi for the most part contained mucus. The largest cyst measured 10 by 3 cm. in diameter. Extending from 1 cm. distal to

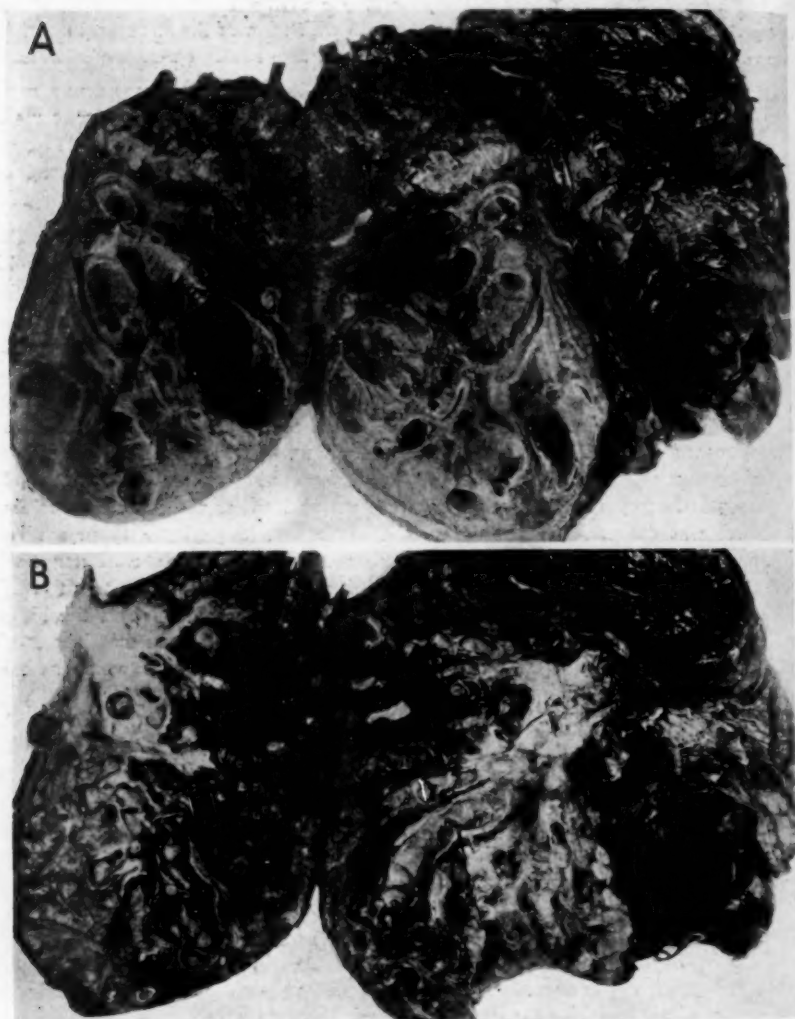


Fig. 4 (case 2).—*A*, sagittal section of the lower lobe of the left lung just beyond the plane of the main tumor, showing the characteristic picture of congenital cystic disease. *B*, sagittal section of the lower lobe of the left lung through the plane of the main tumor, slightly enlarged to show extensive bronchial dilatation and cyst formation.

the point of section of the major bronchus outward for a distance of 8 cm. was a mass of carcinomatous tissue completely occluding the bronchus and apparently having its origin not only from the wall of the primary bronchus but from the walls

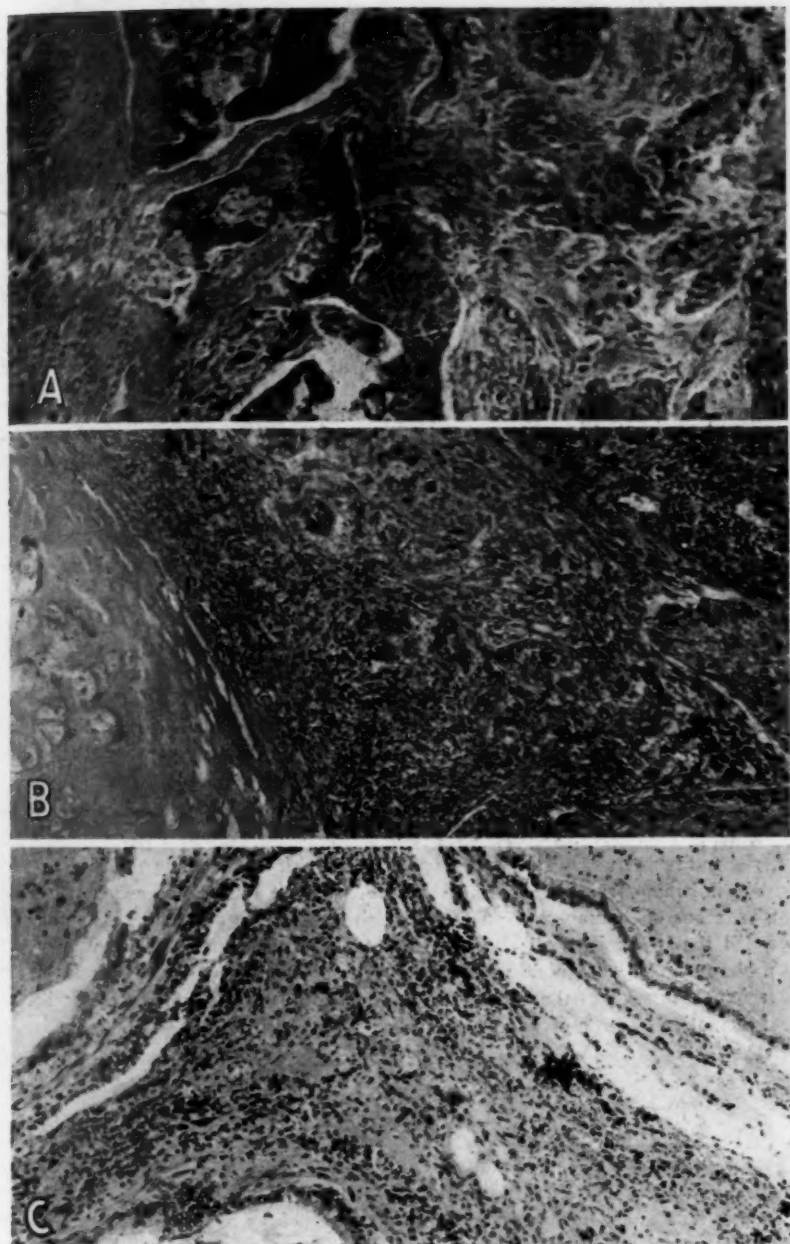


Fig. 5.—Microscopic appearance of the tumor seen in figure 4. In *A* the cells are growing in a dense stroma and show a tendency toward the formation of keratin. In *B* tumor cells are extending between the cartilage rings in a major bronchus. This type of extension usually is associated with involvement of regional lymph nodes. *C* is a section taken from the periphery of the lung, showing multiple cyst formation.

of many of the secondary and tertiary bronchi as well. In spite of the apparently obvious bronchial occlusion, the air sacs between the dilated bronchi seemed well aerated. Sections through the upper lobe showed little gross abnormality.

Microscopic Appearance of Involved Lung.—Figure 5A illustrates the morphologic characteristics of the cancer. It is squamous in type, presenting a definite tendency toward hyalinization with keratin formation in many areas. The stroma is dense. Figure 5B shows invasion between the bronchial cartilages in the primary bronchus with spindle-shaped cells and numerous mitotic figures. Figure 5C is taken from near the periphery of the lung and shows a cluster of cystlike dilatations of the terminal bronchi. The cells are columnar and are both ciliated and goblet in type. There is present a small amount of smooth muscle. These bronchi are filled with mucus and show but little evidence of infection. The adjacent air sacs are collapsed, but nowhere are there any considerable pressure effects. The bronchi are many times the normal size of bronchi in this area and the epithelium is columnar rather than flat or cuboidal, suggesting no great degree of intraluminal pressure.

CASE 3.—A white man aged 52 was admitted to Barnes Hospital Feb. 6, 1941. He had been in excellent health until one year previously, when he began to notice gradually increasing fatigability. Four months before his admission he noticed the appearance of dyspnea, for which he consulted a physician in Buenos Aires, Argentina, where he was living at that time. On the basis of the clinical picture and roentgen studies, this physician made the diagnosis of bronchiogenic carcinoma in December 1940 and referred the patient to St. Louis. There had been a loss of 15 pounds (6.5 Kg.) during the past two years. He had been a heavy smoker and had had a moderately productive cough for the past thirty-five years. The cough had shown no tendency to increase in the past year. On one occasion, however, there had been blood-streaked sputum. There had never been frank hemoptysis, pleurisy or thoracic pain.

Feb. 12, 1941 the entire right lung was removed (E. A. G.) with separate ligation of the vessels and bronchus. A half hour after operation the patient died suddenly from cerebral embolism.

Gross Appearance of Specimen.—The material examined consisted of the entire right lung (fig. 6A). There were old scars and adhesions at the apex. The interlobar fissures were difficult to identify as a result of either incomplete formation or old inflammatory obliteration. On sectioning the lung there could be seen in the bronchus of the upper lobe a firm pinkish mass which obstructed the lumen and invaded the bronchial wall and the surrounding lung tissue. There was present a peribronchial lymph node which showed gross evidence of early carcinomatous invasion. Throughout the upper lobe there were cylindric dilatation of the bronchi and formation of cysts of varying size.

Microscopic Appearance of Involved Lung.—Figure 6B shows an early metastasis in a lymph node and illustrates the morphologic character of the carcinoma present. The cells are both polygonal and spindle shape and in some places show a tendency to form cystlike cavities. Mitoses are frequent, although the variation in nuclear size and shape is not marked. Figure 7A presents a picture that was observed in many areas of the primary bronchus. There is metaplasia with considerable mitotic activity not only of the lining epithelium of the bronchus but of that of the ducts of the mucous glands as well, and in some places (fig. 7B) cancer can be seen having its origin both from the lining epithelium of the bronchus and from the ducts. This cancer shows no morphologic difference depending on which of these structures gave it origin and suggests the fallacy of the grouping of cancers arising from ducts in a separate class from those arising from the lining epithelium. This specimen also offers evidence that cancer does not necessarily always have its

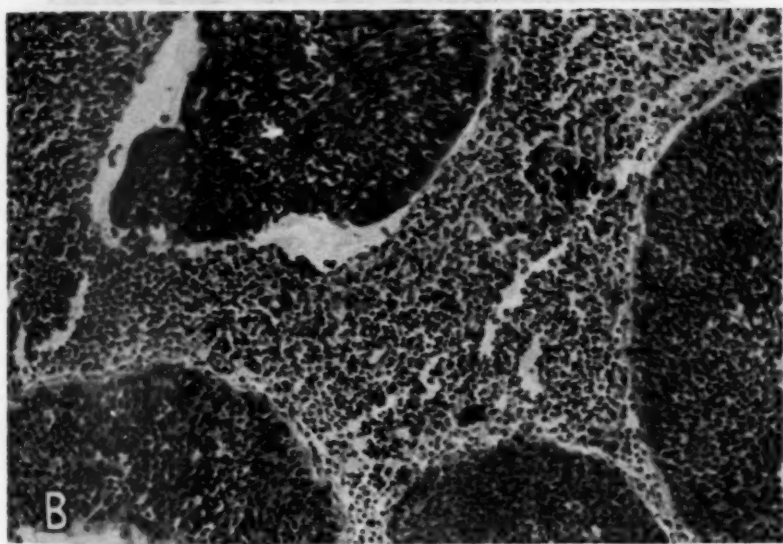
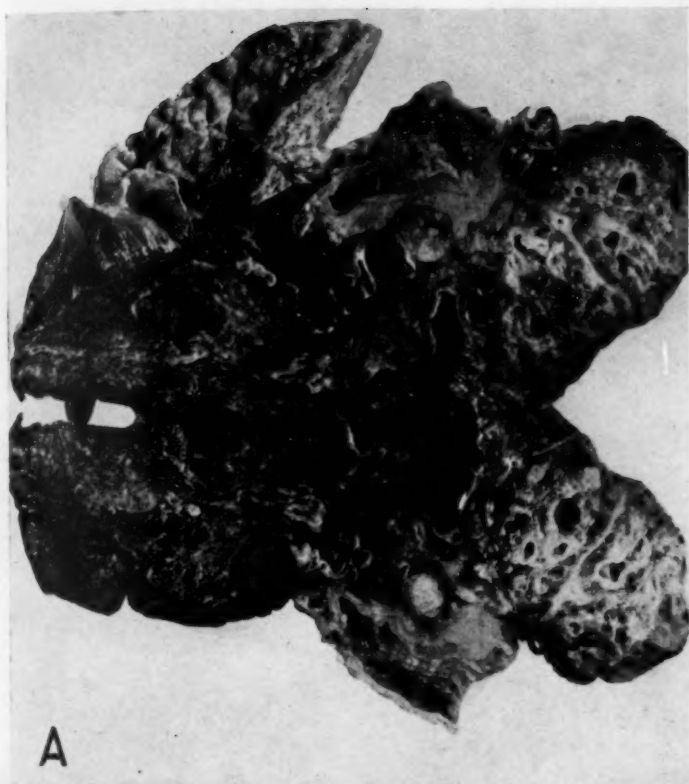


Fig. 6.—*A*, sagittal section through the right lung in case 3, showing tumor occluding the upper part of the main bronchus. Note the cystic dilatation, the failure of complete fissure formation and the involvement of a lymph node.

B, microscopic appearance of a metastasis in the lymph node seen in *A*. Note the spindle-shaped cells and the tendency toward cavity formation.

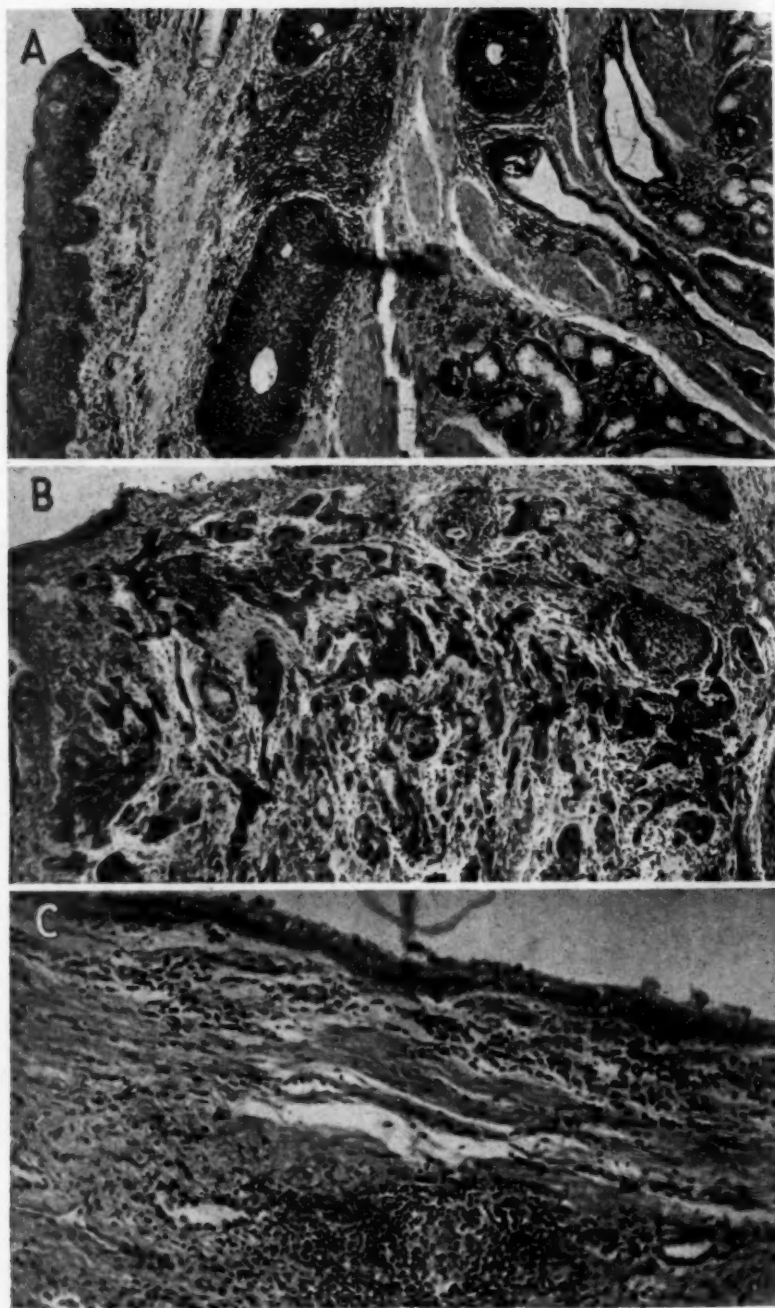


Fig. 7.—*A*, characteristic picture of the involved main bronchus in case 3. Note the metaplasia of lining epithelium in both the bronchus and the ducts.
B, cancer being formed from the lining epithelium of both the ducts and the bronchus. This was some distance from the main tumor.
C, characteristic picture of congenital cystic disease seen in tissue away from the tumor in case 3.

origin from a single focal point but that large areas of pulmonary tissue may undergo malignant change and that these areas are not necessarily adjacent to each other. Bronchiogenic carcinoma thus may be multicentric in origin. The section shown in figure 7C is taken through the wall of one of the larger cysts and shows the characteristic picture seen in congenital cystic disease of the lung.

CASE 4.—A white man 45 years of age was admitted to Barnes Hospital Nov. 6, 1939 with complaints referable to the chest. He first became aware of dyspnea



Fig. 8 (case 4).—Cancer occluding the bronchus to the upper lobe of the left lung. Note the numerous cysts with tumor tissue growing in the formation of cystadenoma.

early in May 1939. This followed severe exertion, but later on dyspnea was noticed after only slight exertion. There was no coughing, pain in the chest or hemoptysis. He was thought to have asthma by his physician, who found him to be allergic to a number of substances. Elimination of these allergens, however, did

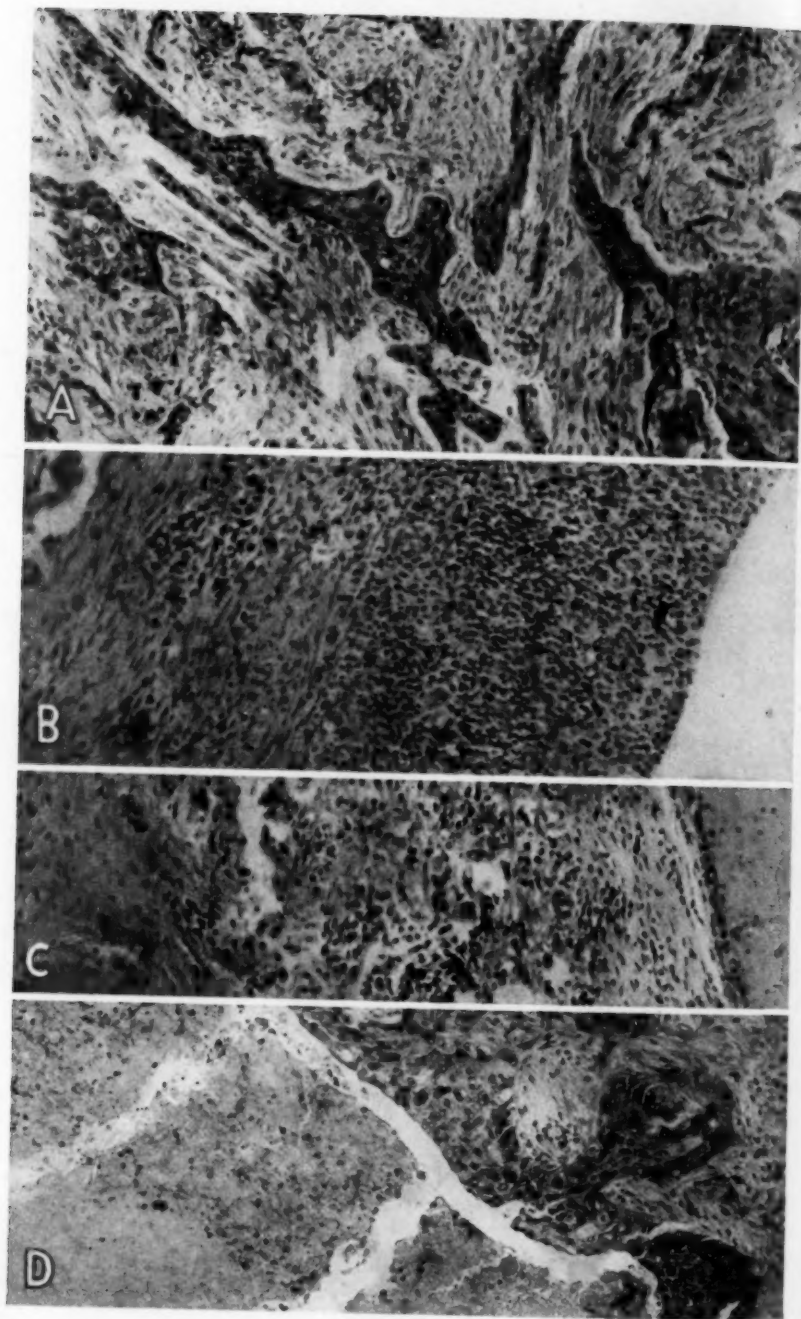


Figure 9

(See legend on opposite page)

not relieve the patient. On the evidence of a roentgenogram of the chest taken at this time, his condition was diagnosed as pulmonary tuberculosis. He remained in bed for five weeks, after which time wheezing respiration ceased. Another roentgenogram was taken which showed atelectasis of the left lung. This was thought to be due to a foreign body, and the patient was treated by bronchoscopy on three occasions with symptomatic improvement. He then received roentgen therapy three times weekly from August 27 to October 20. There was at first a gain in weight of 13 pounds (6 Kg.), which with the appearance of nausea, vomiting and coughing was soon lost. The cough at first was productive of mucus and later of pus. There was no hemoptysis. Examination of the sputum and guinea pig inoculation failed to reveal tuberculosis.

The remainder of the patient's history was irrelevant to the present complaint.

On admission to the hospital there was noticed a respiratory lag at the apex of the left lung, together with deepening of the supraclavicular and infraclavicular fossae on that side. There was dullness to flatness over the left side of the chest anteriorly and posteriorly and particularly at the apex of the left lung. Coarse rales were present throughout the left lung, greater in the apex. There was a decrease in the transmission of both whispered and spoken voice sounds throughout the left side of the chest and particularly at the apex of the left lung. Chemical, serologic and cytologic examination of the blood showed that it was normal. Urinalysis gave normal results. The electrocardiogram was normal. The vital capacity was 2,000 cc.

Bronchography revealed good filling of the left main bronchus and of several divisions of the bronchus to the upper lobe of the left lung. One of the main divisions of this bronchus, however, appeared to be occluded at its point of origin, and there was also distortion in the relationship of the left main bronchus in that its distal end was curved upward and laterally. There was good filling of the lower lobe division, in which there was cylindric bronchiectasis.

A bronchoscopic inspection was made on two occasions, at which time the lumen of the bronchus to the upper lobe of the left lung appeared to be occluded by outside pressure. Biopsy showed only chronic inflammation.

November 20, total left pneumonectomy was performed (E. A. G.). The post-operative course was uneventful except for the necessity of draining accumulated fluid through an intercostal tube. The man was discharged on December 31.

In February 1942 cough began to develop and a bronchial fistula appeared at the site of the previous intercostal drainage. Bronchoscopic biopsy taken from the bronchial stump showed the presence of cancer.

Gross Appearance of the Specimen.—The material examined consisted of the entire left lung (fig. 8). Both lobes were intact, and it had been cleanly removed

EXPLANATION OF FIGURE 9

A, characteristic tumor tissue in case 4. This picture is that of poorly differentiated squamous epithelium growing in a dense stroma.

B, wall of a cyst in case 4, the epithelium of which has been destroyed by the inflammatory process. Note the sharp demarcation and the lack of necrosis, which serve to differentiate it from the wall of an abscess.

C, wall of a cyst in case 4 showing a moderate amount of inflammation and a lining of a single layer of columnar epithelium.

D, lining epithelium of a cyst in case 4, which has undergone squamous metaplasia and has begun to invade the wall.

at the hilus. The visceral pleura was roughened over the apex apparently from the presence of adhesions. The lung in the region of the apex, felt firm in some areas, cystic in others. On looking down the main bronchus, hard white irregular

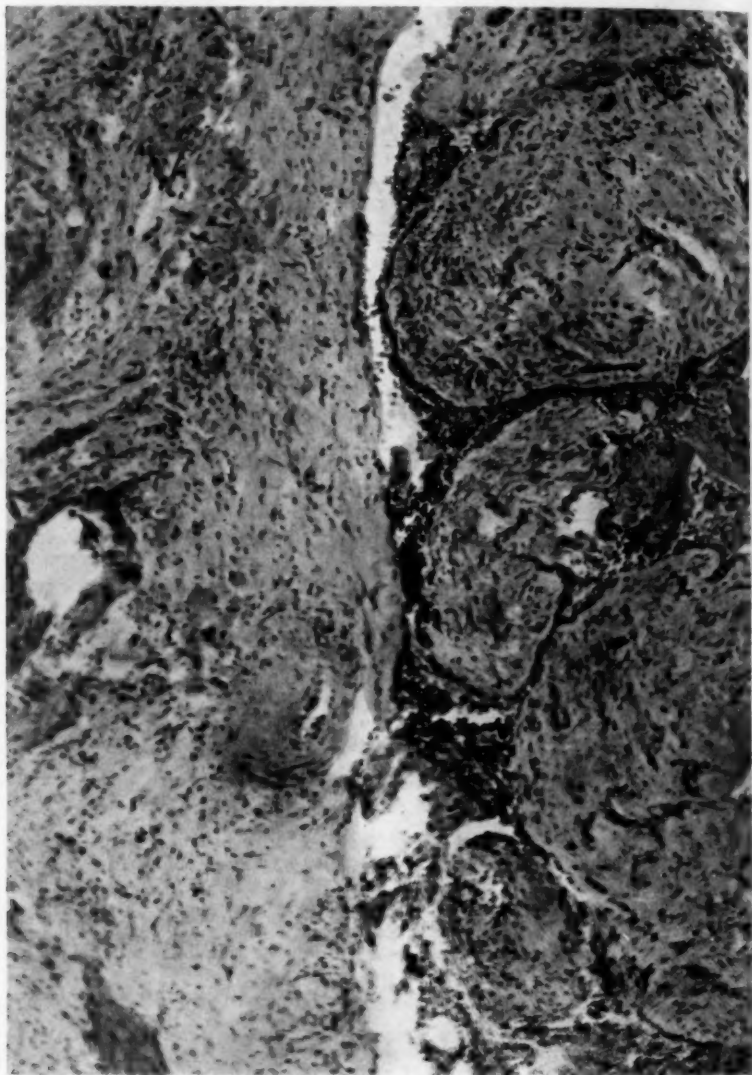


Fig. 10 (case 4).—A nodule of poorly organized lung tissue in which the mesodermal structures predominate, producing a lesion belonging to the group we have designated as "mixed tumors."

tumor tissue could be seen extending upward to about 0.5 cm. from the transected edge. On cut section a hard white tumor involving the main bronchus and the bronchus to the upper lobe was seen infiltrating irregularly into the lung substance,

following in general the course of the bronchus. It extended inward for a depth of about 4 cm., there being no definite outline. The bronchus to the lower lobe was apparently patent, as the entire lobe contained air. In the upper lobe near the apex there were several firm-walled multilocular cavities, the largest of these being 4 by 3 cm. In some of these cavities there was what appeared to be necrotic tissue extending out from the wall, to which it was firmly attached. There was no aerated pulmonary tissue between the walls of these cavities and the pleura, and it was over these areas that pleural adhesions were present. A sagittal section of the entire lung was taken for microscopic study.

Microscopic Appearance of Involved Lung.—Sections taken through the main bronchus showed numerous sheets and groups of malignant squamous epithelial cells growing wildly throughout the tissue. There were pearl formation and destruction and invasion of cartilage, and clumps of tumor cells could be seen in lymphatics. Many mitotic figures were present. In other areas there was invasion of fibrous tissue by cancer cells (fig. 9A). Sections through the cavities showed the walls to be composed chiefly of dense fibrous tissue. In some areas the epithelial lining of the cysts had been destroyed and replaced by inflammatory cells (fig. 9B). In other areas the cysts were lined by a single layer of columnar cells (fig. 9C). In some cysts the lining was hyperplastic and composed of squamous epithelium (fig. 9D), which occasionally definitely invaded through the wall and formed masses of cells extending out into the lumen, with ensuing necrosis. This was the composition of the tissue observed protruding from the walls in the gross specimen. Many of the adjacent lymph nodes in the hilar region were involved with cancer. Near one of the main bronchi there was a lobulated growth resembling the type of lesion we have designated as "mixed tumor." It was approximately 2 mm. in diameter and was composed of epithelial and fibrous tissue chiefly, with an effort at immature bronchus formation (fig. 10).

COMMENT

These specimens represented various types of lesions. In case 1, while the symptoms were undoubtedly produced by the tumor obstructing the main bronchus, independent tumors were likewise present in several of the cysts. The latter lesions apparently had their origin from the lining epithelium of the cysts, giving an appearance similar to that seen in cystadenoma of other organs. While there was no evidence of extension to the regional lymph nodes or metastasis to distant points, the evidence of local invasion was sufficient for one to consider this a cancer. The case illustrates the difficulty in deciding just when a pulmonary tumor of a type thought to be benign becomes cancerous—a question, we feel, long since void of theoretic justification. Morphologically, the cellular picture here was atypical and of a type that might easily be confused with that of tumors of renal origin.

Case 2 was that of a lung in which there are cylindric bronchiectasis and cystic disease but in which the cancer is typically squamous in type and has its origin in the major bronchus. It is difficult to explain by an obstruction of the major bronchus the picture seen in the terminal bronchioles, shown in figure 8. It is characteristic of the microscopic findings in congenital bronchiectasis, in which the terminal bronchioles end blindly, having no apparent connections with atriums or air sacs.

Sections through the larger cysts showed an epithelial lining of a similar type, with no evidence that smooth muscle, glands or cartilage had ever been present.

Case 3 presents evidence of a condition described previously by Lindberg⁴ in which the mucosa of the major bronchus exhibits in many areas a so-called precancerous appearance. It likewise demonstrates that there is usually no morphologic difference in cells between bronchiogenic carcinoma arising from the mucosa and that arising from the glandular ducts. We have called attention to this before. In this specimen the cancer gave the appearance of being multicentric in origin.

Case 4 illustrates the various changes that may be encountered in the walls of cysts in the same lung. Such cysts when filled with mucus and tumor tissue, much of which is necrotic, may superficially resemble abscesses and undoubtedly in times past we have considered them as such. It is only when the entire lung is sectioned and studied microscopically that the actual structure is disclosed. While differing in cellular morphology, this last specimen resembled the first in many respects.

The microscopic pictures presented by these tumors illustrate the pitfalls that will be encountered in any classification of pulmonary cancer based on the shape of the cell. The epithelium of the lung possesses many potencies, and tumors arising from it will present cells of varied structure, the significance of which at the present time is certainly obscure. The rationale, therefore, of classifying cancer of the lung because of a particular cellular shape or cellular function appears to us to be of questionable value.

That primary cancer of the lung may have its origin in areas of developmental disorganization is illustrated in these cases. The site of development may be in several foci at approximately the same time and not limited to cancerous alteration in a single cell or a single group of cells. Such an observation is also suggestive that the preservation of embryonic potencies in maldeveloped tissue, particularly as related to the presence of intracellular enzymatic functions, may play an important role in the development of cancer, and that susceptibility to cancer is intimately concerned with tissue differentiation.

SUMMARY

Four cases are presented of primary cancer of the lung associated with developmental pulmonary abnormalities of different types.

The difficulty of morphologic classification of cancer of the lung is illustrated, and the lack of any significance of such a classification at the present time is emphasized.

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4. Lindberg, K.: *Arb. a. d. Path. Inst. d. Univ. Helsingfors* 9:1, 1935.